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DIEBACK OF BALSAM FIR IN ONTARIO[1]

F. L. RAYMOND AND J. REID[2]

Abstract

An outbreak of balsam fir dieback in Ontario, which was first reported in 1954, reached its peak throughout the Province in 1955 and gradually receded during subsequent years. The dieback, which involved leaders, branches, and occasionally entire trees, appeared when those parts died as the result of their proximal invasion by a fungus. Cankering usually accompanies necrosis; fruiting bodies may be produced immediately or may not appear until the spring following canker formation. The three fungi most commonly found in association with dieback, *Thyronectria balsamea* (Cke. & Pk.) Seel., *Dermea balsamea* (Pk.) Seav., and *Valsa abietis* Fr., were shown to be independently capable of inducing typical dieback and cankering when inoculated into wounds on transplanted trees. It was further shown that adult balsam fir borer beetles (*Monochamus* spp.), which regularly feed on and injure twigs and branches of healthy trees, usually carry viable material of one or more of the three common fungi. The ability of the beetles to kill twigs was confirmed and it was shown that, although dieback frequently develops from wounds made by fungus-infested beetles, no dieback ensued in the absence of fungi. The outbreak is believed to have been caused by the interaction of several factors (climate, fungi, site, and insects) on the host trees.

Introduction

Numerous dead balsam fir trees (*Abies balsamea* (L.) Mill.), conspicuous because of the redness of their needles, were observed in widely separated areas of Ontario and Quebec during 1954 (24). The greatest mortality of entire trees undoubtedly had occurred in Quebec (15). In Ontario, where the condition was called "canker and dieback", usually no more than the upper one-half to three-quarters of each affected tree had died, the basal portion remaining alive and green. The margin between the living and the dead portions of such a tree usually coincided with the proximal edge of a canker which had been formed as the result of shrinkage of dying and drying bark (19). Similar, but less extensive, cankering of branches and leaders was also noted in New Brunswick during 1954, but no tree mortality was reported (7)

In 1955 in Ontario, an extensive survey demonstrated that the condition had become common throughout most of the range of balsam fir in the Province and that it had become more severe. Affected trees were no longer mainly

[1]Manuscript received October 24, 1960.
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confined to roadsides or to other extremely exposed situations. Many of them were found to be dead throughout; some had been killed by the extension of previously existing cankers, others without having been previously affected. Also, in 1955, a fungus, *Thyronectria balsamea* (Cke. & Pk.) Seel., was found to be abundantly associated with the cankers on the majority of affected trees (12, 20).

During 1956 and subsequent years, two additional fungi, *Dermea balsamea* (Pk.) Seav. and *Valsa abietis* Fr., were found on many of the affected trees examined by the authors. Furthermore, it was observed that the cankers were frequently associated with wounds, common among which were those characteristic of the scars made by adult borer beetles (*Monochamus* spp.) when feeding on healthy, living branches. These findings, together with the results of preliminary experiments involving the inoculation of balsam fir with isolates of these three fungi, had led the authors to publish a note in which it was theorized that most of the dieback of balsam fir in eastern Canada probably resulted from the combined activities of fungi and insects. The latter were believed to provide entry courts for infection, to inoculate mechanically produced wounds, or to both wound and inoculate the trees (21).

The present paper is the result of continued studies on the relationships of these three fungi and of *Monochamus* spp. to balsam fir dieback. The probable roles of these organisms in dieback are discussed and the possible influence of climate and site is also considered.

Symptomatology and Epidemiology

Balsam fir dieback is manifested by the dying of all or part of a tree proximal to the point of infection or to a previously dead portion. The part which dies has been invaded by an infecting fungus or has been cut off by the girdling action of such an invasion. Frequently, this invasion can be traced to a wound through which the fungus apparently had entered. Any type of wound may provide entry for the causal organism, but non-mechanical wounds typical of the feeding scars made by adult borer beetles (Fig. 3) are often found associated with dieback. Death of the branches or leaders may keep pace with their fungal invasion or may not occur until the following spring. A newly dead portion of a tree is characterized by the following symptoms: any tender tissues have wilted, all of the needles have become strikingly reddened, and the bark and outer sapwood have dried and shrunken with the result that a canker has been formed. Fruiting bodies may be produced by the invading fungus immediately after necrosis; however, they may not appear until the following spring.

The extent of dieback is partly determined by host size. Trees larger than 6 in. in diameter, breast height, usually do not experience appreciable dieback. Trees which are killed in a single season rarely exceed 5 ft. in height. The death of any stem larger than 1 in. is more likely to have resulted from its having been girdled by fungi invasion through the base of an infected branch than from the proximal extension of an infection in the leader.

The reddened needles of affected trees usually remain attached for several years but lose some of their color with each overwintering. Therefore, it is frequently possible to distinguish between those portions of a tree which had

died in different years, and comparisons can be made between the annual rates or extents of invasion; thus, any trends or major changes in rate can be visually determined. Examinations of color gradations associated with naturally occurring dieback revealed that there had been a marked general decline in the extent of invasion during the period 1955 through 1959. During that same period there also had been a decrease in the incidence of the disease. Exceptions to these tendencies have usually been associated with disturbances such as logging or road-building. In the latter case, for example, there frequently is damage and increased exposure of trees at the margins of rights-of-way. Both of these conditions result in the lowering of the vigor of those individuals for a few years, or in their death. Accumulations of dead and dying trees favor the multiplication of borers.

In areas where no recent disturbances had occurred, most of the trees affected by dieback were found on shallow or excessively drained sites and in very exposed situations, such as roadsides or the edges of openings. Trees in protected situations or on better sites usually were affected only during and immediately following years of unfavorable climatic conditions.

Causal Organisms

In 1955, Quirke and Hord (12, 20) had found an abundance of *Nectria*-like perithecia in association with the cankered bark on most of the affected trees. They believed that this fungus, which was subsequently determined by Hord[3] to be *Thyronectria balsamea* (Cke. & Pk.) Seel., probably had caused the disease.

By examining the specimens of cankered balsam fir on which their 1955 reports were based, the authors were able to confirm the great frequency and abundance of *T. balsamea* fruiting bodies and to obtain cultures from some of them. Also, a search of the related collection records indicated that of the 144 samples of dieback which had been received at Maple during 1955, 114 had borne signs of this fungus. The identification was confirmed by comparison with the specimens on which Seeler had based his concept of that species in his monograph of the genus *Thyronectria* (22). These specimens were kindly lent to us by Dr. I. M. Lamb, Director of the Farlow Herbarium at Harvard University.

Certain pertinent characteristics of *T. balsamea* are appended here in the hope that they will be sufficient to distinguish it from other fungi which grow on balsam fir, and that their inclusion will help to eliminate some of the recurrent confusion of its identity. The perithecia of *T. balsamea* are gregarious; 10 or 20 may occur on each stromal pad. Both structures are predominantly orange in color. Each ascus usually contains three or four ascospores which are variable in size (7–30 × 3–5 μ), in shape (subcylindrical to fusiform), and in septation (irregularly muriform). Apparently within a very short time after these spores have been formed, they begin to bud off bacillar secondary ascospores or ascoconidia which fill each ascus (Fig. 2) and which are the spores commonly extruded through the ostioles in gelatinous masses during wet or humid weather.

[3]Standard Fruit and Steamship Co., La Ceiba, Honduras. Personal communication.

In 1956, it was first noticed that, although *T. balsamea* was the fungus which was most consistently associated with the cankers of trees affected by dieback, two others occurred with noteworthy frequency. The more common of these was *Dermea balsamea* (Pk.) Seav., usually represented by the *Gelatinosporium* stage, *G. abietinum* Pk.; the other was *Valsa abietis* Fr., usually present in the *Cytospora* stage, *C. abietis* Sacc. The identities of these fungi were determined by comparison with specimens from the mycological herbarium of the University of Toronto, which were kindly lent to us by Prof. R. F. Cain.

The fruiting bodies of *G. abietinum* are non-osteolate pycnidia. Each is immersed in a small individual stroma and is yellow or yellowish green when young, but erumpent and black at maturity when it splits open irregularly. The simple or slightly lobate cavity contains predominantly simple conidiophores. The conidia are hyaline; they are unicellular and acerose when young (microconidia (10–20 × 1–2 μ), and may be one- to four-celled and falcate at maturity (50–90 × 3.5–5 μ). They are extruded in mucilaginous masses.

Mature fruiting bodies of *C. abietis* are erumpent, brownish, multilocular stroma, 500–700 μ in diameter. All of the locules in each stroma open into a common central ostiole through which the hyaline, allantoid spores (4–5 × 1 μ) are extruded in yellowish-orange tendrils.

Observations, Experiments, and Results

Pertinent observations, together with related experiments and their results, are presented in Sections A to F.

A. Inoculation of Potted Balsam Fir with *Thyronectria balsamea* under Laboratory Conditions

Observations made during the 1956 field season clearly demonstrated that *T. balsamea* was still the fungus most commonly found on the cankers of affected trees. Consequently, a test of the pathogenicity of this organism was undertaken in January, 1957. Twelve apparently healthy young trees ranging from 24 to 30 in. in height were brought to Maple from the University of Toronto Experimental Forest near Dorset, Ontario. After having been root-pruned and individually potted, they were kept on a laboratory bench in a humidified room. In addition to the daylight which reached them through the windows, they were provided with artificially extended "days" made up

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Fig. 1. Artificially wounded balsam fir twigs from potted trees. The twig on the right was inoculated with *Thyronectria balsamea*. Note that the canker has extended below the wound and that all needles distal to it are red.

Fig. 2. Primary and secondary ascospores of *T. balsamea*. Both are produced in the ascus. × 720

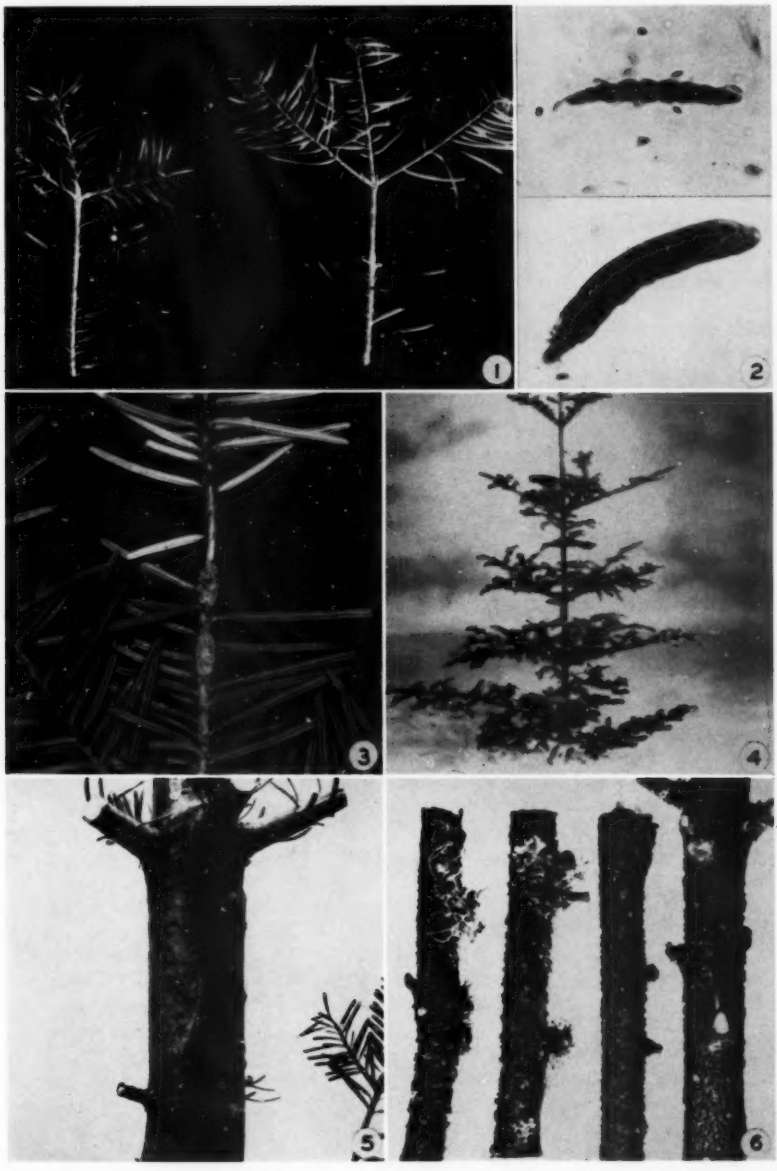
Fig. 3. Twig with scars resulting from feeding by adult *Monochamus* beetles during three successive years. The lowest scar is the earliest. Only the latest feeding was sufficiently damaging to cause twig killing.

Fig. 4. Affected tree with living basal branches. Immediately above them is the base of the stem canker.

Fig. 5. Typical stem canker. Infection entered the stem through the lower lefthand branch-base.

Fig. 6. Sections of dead stems illustrating heavy fruiting by *T. balsamea*.

PLATE I



Raymond and Reid—Can. J. Botany

of two light periods, totalling 15 hours out of every 24. The major period of 14 hours overlapped the natural day at both ends and the minor period of 1 hour came in the middle of the natural night. The artificial illumination was produced by a bank of five 24-in. daylight-type fluorescent tubes interspersed with four 18-in. 60-watt incandescent tubes; the total intensity was approximately 300 foot-candles at crown level. The lights were turned on and off at appropriate times each day by an electrical timer. Room temperature usually remained near 76° F when the lights were on and near 70° F during the periods of darkness.

After 10 days, nearly all of the lateral buds on 5 of the 12 potted trees had broken dormancy. After 3 weeks, when their new shoots were growing vigorously, seven similar branches on each of four of these trees were selected and treated as follows: three of the seven branches on each tree were wounded and inoculated, two were inoculated without wounding, one was wounded but not inoculated, and one was neither wounded nor inoculated. Each wound was made at a point about 4 in. from the tip of a branch by slicing from its upper surface a strip of bark, cambium, and outer sapwood that was $\frac{1}{2}$ to $\frac{3}{4}$ in. long by about $\frac{1}{8}$ in. wide. Inocula consisted of agar disks about $\frac{3}{8}$ in. in diameter, each with fungal mycelium on one side of it. Each disk was cut from the margin of a 9-day-old culture of *T. balsamea* which had been grown on a plate of modified Hagem's agar (malt extract, 5 g; glucose, 5 g; KH_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; NH_4Cl, 0.5 g; $FeCl_3.6H_2O$, 0.5 ml of a 1% solution; V-8 vegetable juice, 200 ml; H_2O, 800 ml; and agar, 40 g). A disk was applied mycelial side down, at the appropriate point on each branch selected for inoculation, and was held in place by means of several turns of sterile gauze. Each selected branch, whether inoculated or not, was enclosed in a small plastic bag containing about 10 ml of sterile, distilled water. Uninoculated branches received neither disk nor gauze prior to being bagged. All bags and gauze windings were removed 4 days later. The fifth tree was retained as an untreated control.

Four weeks later, infections had developed in 8 of the 12 branches which had been both wounded and inoculated, and in 1 of the 8 branches which had been inoculated without wounding. No infections were found in the four wounded or the four unwounded branches which had not been inoculated; neither were any infections found in the untreated tree. A typical infection is illustrated by the right-hand branch in Fig. 1. At this time small orange-red perithecial initials had begun to form on many of the cankers, and *T. balsamea* was reisolated from all parts of all infected twigs which were sampled.

These results demonstrated that, under the conditions of the experiment, *T. balsamea* was capable of acting as a wound parasite and inducing dieback. The symptoms which were produced were similar to those frequently found in nature.

It should be noted that six of the seven trees which remained dormant were dead within 2 months after having been potted. They declined and became desiccated in spite of a reasonable supply of water. In four of those six trees, the shrinkage which resulted from drying revealed the presence of abundant pycnidia of *Gelatinosporium abietinum* embedded in the bark along their stems and branches.

B. Inoculation of Transplanted Balsam Fir under Field Conditions

Transplanted trees were employed in the second inoculation experiment. Apparently healthy young trees averaging about 30 in. in height were dug from within the University of Toronto Experimental Forest early in April, 1957, and planted in a cool, moist, protected situation on the grounds of the Southern Research Station near Maple, Ontario. This was done to assemble the trees in an accessible area which was expected to be relatively free of the organisms commonly associated with balsam fir dieback, since the host was absent from the vicinity. Late in June, when the transplants appeared to be well established and were growing vigorously, a number of them were employed in an experiment involving inoculations with mycelia of *Thyronectria balsamea*, *Dermea balsamea*, and *Valsa abietis*. Later, other well-established transplants from the same lot were subjected to an experiment which involved inoculations with spores of *T. balsamea* or *D. balsamea*.

Mycelial Inoculation

In this experiment, 84 of the vigorously growing transplants were wounded and inoculated with the three test fungi, singly or in combination. Twenty additional trees, of which 10 were wounded and 10 were not, served as controls. Except for the 10 unwounded controls, every tree received five wounds per organism prior to inoculation. One of every five wounds which was prepared for *T. balsamea* was made about two-thirds of the way up the stem; each of the other four was made about 4 in. from the tip of a branch. Preparation for each of the other fungi was made by wounding five branches, each at a point about midway between its tip and the stem. An attempt was made to select for wounding, branches which were evenly distributed throughout each crown.

Mycelial inocula of the three fungi were grown and applied as in the experiment with the potted trees. Here, however, each agar disk was wrapped with moist, sterile cotton held in place by a small sheet of aluminum foil twisted to clasp the branch firmly at both sides of the cotton. Foil and cotton were removed 1 week later.

Certain differences in pathogenic activity were soon apparent. About $\frac{1}{10}$th of the *T. balsamea* inoculations resulted in wilting, needle reddening, and cankering within 9 days, regardless of whether those trees had received mycelia of the other fungi. With the other $\frac{9}{10}$ths of the wounds which had received *T. balsamea*, symptoms of tip killing and dieback appeared more slowly, but most of such tips which died did so within 6 weeks after inoculation, although many of the affected branches did not develop dieback until the following spring. Symptoms caused by infections of the other test fungi usually began to appear much later in the season; frequently, they did not appear until the following spring.

The results of infections one year after inoculation are summarized in Table I. These results indicate that, under the experimental conditions, all three test fungi were able to initiate dieback in wounded branches. Apparently, *T. balsamea* is the fastest acting of the test fungi and may be the most virulent.

TABLE I

Results 1 year after field inoculations of balsam fir transplants with mycelia of *Thyronectria balsamea*, *Dermea balsamea*, and *Valsa abietis*

Fungus	No. trees	Preparation of trees	Results		
			No. trees dead	No. with dieback	No.* without dieback
T	14	Wounded	4	8	2
D	11	"	0	7	4
V	11	"	0	4	7
T, D	12	"	2	9	1
T, V	12	"	4	6	2
D, V	12	"	2	7	3
T, D, V	12	"	4	5	3
None	10	"	0	2	8
None	10	Unwounded	0	0	10

*Includes branches with dead tips which did not give rise to dieback.

Spore Inoculations

Naturally occurring infection and dieback may originate more frequently by spores than by mycelia. Consequently, the ability of spores of *T. balsamea* and *D. balsamea* to infect wounded or unwounded branches was tested on 27 additional, well-established transplants early in August. On 13 of the transplants, five branches per fungus were wounded at their midpoints; 14 transplants were not wounded. Ten comparable trees, five of which were wounded and five not, were retained as controls. Aqueous suspensions of spores of each fungus were prepared by obtaining the spores from fruiting bodies on freshly collected samples of dieback which had been placed in moist chambers. Aliquots of these suspensions were sprayed on the wounded or unwounded branch midpoints of the 27 test trees. These loci, as well as comparable ones on the control trees, were individually wrapped with moist cotton and sealed with foil, as in the previous experiment. Cotton and foil were removed 1 week later.

TABLE II

Results 1 year after field inoculations of transplants with spores of *Thyronectria balsamea* and *Dermea balsamea*

Fungus	No. trees	Preparation of trees	Results		
			No. trees dead	No. with dieback	No.* without dieback
T	5	Wounded	5	0	0
D	5	"	2	3	0
T, D	3	"	3	0	0
T	5	Unwounded	0	1	4
D	5	"	0	2	3
T, D	4	"	0	1	3
None	5	Wounded	0	0	5
None	5	Unwounded	0	0	5

*Includes branches with dead tips which did not give rise to dieback.

The results of infection 1 year after inoculation are summarized in Table II. Clearly, spores of either *T. balsamea* or *D. balsamea* were capable, under the existing experimental conditions, of causing dieback when sprayed on wounds on branches of the transplanted trees. Similar inoculation of unwounded branches less frequently resulted in dieback.

C. Inoculation of Naturally Grown Balsam Fir in the Field

Late in May, 1958, shortly before the breaking of dormancy, 25 naturally occurring and apparently healthy young balsam fir trees, which averaged about 3 ft. in height, were selected in each of two areas. The first site was a dry sand plain near Highway 11, a few miles north of Bracebridge, Ontario. Here, the young balsam constituted the predominating reproduction under a few immature eastern white pine (*Pinus strobus* L.) at the edge of a large opening. The other site was a moderately deep, fresh till within the University of Toronto Experimental Forest. Here, the young balsam were scattered among the advanced reproduction of hard maple (*Acer saccharum* Marsh.) and other deciduous species under an open canopy of immature hard maple and yellow birch (*Betula alleghaniensis* Britt.).

Twenty of the 25 trees selected at each location received five wounds each, one on the leader and the others at the midpoints of four branches distributed throughout the crown. The wounds of five trees in each area were inoculated with *T. balsamea*, *D. balsamea*, or *V. abietis*. The inocula were prepared and applied as in the previous experiments employing mycelial disks. Each disk of agar and mycelium was held in place for 1 week with moist, sterile cotton and aluminum foil.

The results 1 year after inoculation are summarized in Table III. No dieback had been induced by any of the inoculations. However, it should be pointed out that on both of the areas involved, nearly 50% of the inoculations with *T. balsamea* had resulted in well-established infections with

TABLE III

Results 1 year after field inoculations of naturally occurring balsam fir trees with mycelia of *Thyronectria balsamea*, *Dermea balsamea*, or *Valsa abietis*

Area	Fungus	No. trees	Preparation of trees	Results		
				No. trees dead	No. with dieback	No. with definite infections
Bracebridge	T	5	Wounded	0	0	4
	D	5	"	0	0	2
	V	5	"	0	0	0
	None	5	"	0	0	0
	None	5	Unwounded	0	0	0
Dorset	T	5	Wounded	0	0	4
	D	5	"	0	0	2
	V	5	"	0	0	0
	None	5	"	0	0	0*
	None	5	Unwounded	0	0	0*

*One of these trees had a dead tip associated with *Monochamus* feeding.

distinct symptom expression, even though none of the infections had caused sufficient proximal extension to justify calling it dieback. On the other hand, *D. balsamea* gave rise to obvious infection in only 4 of 50 wound inoculations of trees on the two areas. No apparent infection resulted from attempts with *V. abietis*.

D. Identity of a Very Common Agent of Balsam Fir Injury in Nature

During the 1956 and subsequent field seasons, the authors noticed that a particular type of little scar could frequently be found on small branches and twigs of affected trees in which the symptom expression varied from substantial cankering and dieback to tip killing only. However, the origin of these scars remained unknown to us until the middle of the 1957 season, when they were identified as feeding scars made by adult balsam fir borer beetles (*Monochamus scutellatus* Say and *M. marmorator* Kby.).[4] "Red branch" associated with feeding by those beetles was apparently first reported from central Ontario in 1920–1921 (10). Descriptions of the feeding habits and other activities of those species and of related tip-killing were provided in 1924 from studies carried out in eastern Ontario (23). "Red branch" attributed to *Monochamus* feeding was also reported for many points in Quebec and New Brunswick in 1940 (1).

In Ontario, adult beetles usually begin to emerge in late June or July. They feed on the bark and needles of apparently healthy branches throughout the summer. Oviposition may occur within a few weeks of emergence. Gravid females select the bark on trunks of dying or recently dead trees in which to gnaw slits to receive their eggs. One or two years later, the larvae which develop from those eggs will have become adults and will emerge through bark which may be covered with fruiting bodies (Fig. 6). They will then feed upon living trees (23, 4).

E. Isolations of Fungi from Beetle-scarred Branches and from Beetles

Isolations from Beetle-scarred Branches

During the later part of the 1957 field season, 142 twigs or branches with scars which apparently had been made by *Monochamus* spp. were collected and processed. Symptom expression among the samples varied from none (that is, apparently healthy), through needle reddening without bark shrinkage, to branches cankered and dead. When a branch sample arrived at the laboratory, it was first examined for identifiable fruiting bodies of fungi, and their occurrence was recorded. If none could be discovered, the portion bearing the scar was liberally painted with a 1% solution of mercuric chloride in 95% ethanol. After about 1 minute, most of the outer periderm which had been so treated was scraped away aseptically and a number of chips consisting of inner bark, cambium, and outer sapwood were placed on plates

[4]Mr. Angus Harnden, Regional Research Laboratory (Forest Biology), Sault Ste. Marie, Ontario. Personal communication.

of modified Hagem's agar for incubation. One or more of the three fungi under investigation here, *Thyronectria balsamea*, *Dermea balsamea*, or *Valsa abietis*, was isolated from, or found fruiting on, 117 of the 142 samples.

To provide data for comparison, 563 samples of diseased balsam fir were similarly processed in 1958. The presence of any symptom of infection was the criterion for collection, and it was applied without regard to the presence of injuries from any source. In the laboratory, as before, identifiable fruiting bodies were recorded and any sample lacking them was surface-sterilized about the proximal margin of the dead or apparently infected stem tissue. Chips were cut from that margin and plated on malt agar. One or more of the three fungi under study was found fruiting on or was isolated from 397 of the 563 samples.

In both of the series of samples described above, *D. balsamea* was recorded with slightly greater frequency than was *V. abietis*. *T. balsamea* was found approximately twice as often as either of the other two fungi.

Isolations from Monochamus spp.

Two hundred and eighty-six specimens of *M. scutellatus* or *M. marmorator* which had been trapped at various points throughout Ontario were processed in an attempt to determine what fungi they were harboring. Each beetle was treated in one of the following ways: (*a*) shaken at high speed for 15 minutes in sterile, distilled water which was then diluted and plated on malt agar; (*b*) dipped in 95% ethanol and rinsed in sterile water before being treated as in "*a*"; (*c*) dipped in a 20% aqueous solution of commercial Javex and rinsed, then permitted to crawl over the surface of malt-agar plates for varying periods of time; (*d*) immersed in 95% ethanol until dead (about 5 minutes) then rinsed and dissected, the mouth parts, antennae, and legs being transferred to malt-agar plates; (*e*) treated as in "*d*" except that an immersion in 5% aqueous calcium hypochlorite solution and a rinse followed the ethanol immersion and rinse.

The results are presented in Table IV. Nearly 25% of the beetles which had received a surface-sterilization treatment and approximately 67% of those which had not were found to have been carrying viable material of one or more of the three fungi under investigation.

TABLE IV

Results of isolations from 286 specimens of *Monochamus* spp. from various natural sources

Isolation procedure	No. beetles processed	No. yielding: *Thyronectria balsamea*	*Dermea balsamea*	*Valsa abietis*
Washing, plate dilutions	100	40	8	19
Alcohol dip, wash, plating	50	10	1	4
Javex dip, wash, crawl	50	3	0	0
Alcohol dip, dissection	50	2	10	10
Alcohol dip, calcium hypochlorite, dissection	36	1	0	4

F. Inoculations of Transplanted Balsam Fir by Means of Fungus-infested Beetles

In April, 1958, a number of additional young, apparently healthy balsam fir ranging from 24 to 30 in. in height were obtained from the University of Toronto Experimental Forest and planted in a location similar to and about 100 yd from those planted in 1957. Early in July, when the new transplants were well established and were growing vigorously, 36 of them were individually enclosed in cages of galvanized wire mesh and were utilized in the following experiment.

Specimens of *Monochamus* spp. were obtained by placing, in a cheesecloth-covered enclosure, balsam fir logs which were known to contain pupae of these beetles and which were not bearing visible fungal fruiting bodies. Newly emerged beetles were collected each day. Each was surface-sterilized as much as feasible by dipping in 95% ethanol, rinsing, dipping in a 5% aqueous solution of calcium hypochlorite, and washing thoroughly in sterile, distilled water. Each was then infested with one of the three test fungi by placing it in a small moist-chamber containing pieces of bark on which the fungus was fruiting. After the beetle had crawled and fed on the bark and the fruiting bodies for 48 hours, it was transferred to one of the caged trees together with three other beetles of the same infestation history. Eight trees per fungus were so treated. The beetles were left in cages until four to nine feeding scars could be seen on the tree; the period of time usually did not exceed 40 hours. Three control trees received beetles which had been surface-treated but which had not been fungus-infested before they were placed in the cages and allowed to feed. Three other trees served as untreated controls, these receiving no beetles.

At the end of the feeding period, the cages were removed from the trees and the infested beetles were recaptured and returned to the laboratory for isolation of the infesting fungi. *T. balsamea* was recovered from 75% of the beetles on which isolation was attempted, *D. balsamea* from 44%, and *V. abietis* from 67%.

Dead tips began to appear on many of the wounded branches within 3 weeks after treatment. By early October, 3 months after the fungus-infested

TABLE V

Results 3 months after the inoculations of balsam fir trees by means of fungus-infested beetles

Fungus	No. trees	No. scars	No. scarred branches	No. with dead tips	No. with dieback	No. dieback branches cultured	No. yielding inoculated fungus
T. balsamea	8	84	78	74	60	38	27
D. balsamea	8	92	74	68	50	36	24
V. abietis	8	78	68	46	34	34	21
None	3	34	24	14	2	2*	†
None	3	0	0	0	0	—	—

*All 14 branches with dead tips were processed.
†One of the two specimens yielded *D. balsamea*, the other yielded only an undetermined fungus.

beetles had fed on the trees, dieback, resulting from the proximal extension of infections from feeding scars, had affected a number of entire branches. In a few cases where *T. balsamea* was involved, well-defined stem-cankers were being formed. At that time, all branches with feeding scars were removed from the trees and taken into the laboratory for reisolation of the infecting fungi. The results of the feeding, infection, and dieback, and of the attempts to reisolate the test fungi are given in Table V.

The results obtained from the feeding by presumably uninfested beetles are particularly interesting. Note that on the three trees involved, 14 of the 24 scarred branches developed dead tips; two of these gave rise to dieback. Both cases of dieback yielded fungi; *D. balsamea* was isolated from one and an unidentified organism from the other. None of the other 12 dead tips yielded fungi. Clearly, feeding by a beetle can result in the death of that part of the branch distal to the injury, but proximal invasion from that point apparently requires infection by a fungus.

Discussion and Conclusions

From the evidence presented in this paper, it is clear that all three of the fungi which have been found in common association with naturally occurring balsam fir dieback in Ontario are independently capable of inducing typical symptoms of dieback under certain experimental conditions. In descending order of frequency and apparent virulence, they are *Thyronectria balsamea*, *Dermea balsamea*, and *Valsa abietis*; the last two are usually represented by their pycnidial stages, *Gelatinosporium abietinum* and *Cytospora abietis*, respectively. *T. balsamea* may be the entity which was reported, under the synonym *Chilonectria cucurbitula* (Curr.) Sacc., to have caused "burn blight" of jack (*Pinus banksiana* Lamb.) and red (*P. resinosa* Ait.) pines in Michigan and Wisconsin during the period 1940 through 1945 by infecting wounds, especially those made and inoculated by the spittle bug (*Aphrophora saratogensis* Fitch) (11). It may also be identical with the fungus reported as *Nectria* sp. in connection with the recent outbreak of balsam fir dieback in Quebec (16, 17) and with the fungus identified as *Creonectria cucurbitula* which was reported, in 1942, to have caused girdling cankers of balsam fir in northeastern North America (14). In 1952 it appeared during the year after death on certain balsam fir which died very quickly after having been attacked by the woolly aphid (*Adelges piceae* Ratz.) in New Brunswick (3). Such failure of the fungus to appear during the first year of the necrosis was also noted in connection with balsam fir dieback in Ontario (12, 20) and with the pine blight in Michigan and Wisconsin (11).

On the other hand, these three commonly occurring entities are not the only fungi which have been found in association with balsam fir dieback or which are believed capable of causing the cankers related to that malady, either in Ontario or elsewhere. A fourth and still unidentified fungus was found to be locally common in one small area of western Ontario. In 1956, a species of *Godronia* was reported to be causing a repeating leader kill of balsam fir in Quebec (16). *Fusicoccum abietinum* Prill. & Delacr. was reported to have been responsible for at least a part of the branch and leader cankers found

on that host in New Brunswick during 1956 and 1958 (8, 9). It may be the imperfect stage of the Quebec *Godronia* species. *Cephalosporium album* Preuss was believed to have caused the canker and dieback of balsam fir which was widespread in Minnesota and Wisconsin from 1933 through 1935 (5). It was also found causing cankers on the stems of some of the balsam fir which had been artificially wounded in 1937 as a part of a different study in the same area (6).

Of the conditions usually required for the successful induction of dieback by any of these fungi, host injury apparently is important. Although it was shown that either the spores or the mycelia of the three test fungi were occasionally capable of causing infection and dieback of uninjured branches, it is clear that they usually function as wound pathogens. Some of the injuries frequently found in association with naturally occurring dieback are of mechanical origin, but most may have been made by the feeding activities of borer beetles belonging to *Monochamus* spp. It was shown that these beetles are not only capable of providing the wound, but are also capable of inoculating it if they are infested with one of the test fungi. It was further shown that the beetles frequently carry viable material of one or more of those fungi. Although an experiment with presumably uninfested beetles confirmed their ability to cause tip killing, the evidence indicated that dieback of such branches can ensue only after infection.

With regard to the dying of branch tips subsequent to beetle damage, there are indications that such "red branch" results only when one or more additional factors is also present. Faull, who was clearly dealing with tip-killing and not with dieback, believed that the branch ends died only when the interval between damage and the occurrence of autumn frosts was too short to permit the wounds to heal. He noted that 24 of 25 artificial wounds, similar to beetle scars, made in October, 1919, resulted in "red branch" by the following May. Branches on which he had made similar wounds in July, 1919, remained healthy (10). Both our experimental results and our field observations are consistent with that belief. The experiment with caged beetles was terminated only after several weeks of frost. Either the frosts or the shock of transplantation of the trees, or both, could have been important contributing factors in the death of uninfected branch tips. In the field it had been noted that beetle scars very often do not result in "red branch". Furthermore, when damaged branches become affected during the summer they are usually accompanied by wilting of the current season's growth and *Thyronectria balsamea* usually can be isolated. In typical "red branch", from which dieback fails to develop, death occurs during the winter and no succulent foliage is involved.

Dieback is the death of host tissues as the result of a proximally extended invasion of those or of other tissues by an infecting fungus. For example, an infection in a leader may progress downward along the bole and the resultant dieback will involve not only the bole, which was invaded, but also the attached branches, which were not. However, when an appreciable segment of a bole has died, it often has done so as the result of the girdling action of an invasion which reached the bole by proceeding inward along a branch (Fig. 4).

It is not surprising, therefore, that tree size, and more specifically, the size

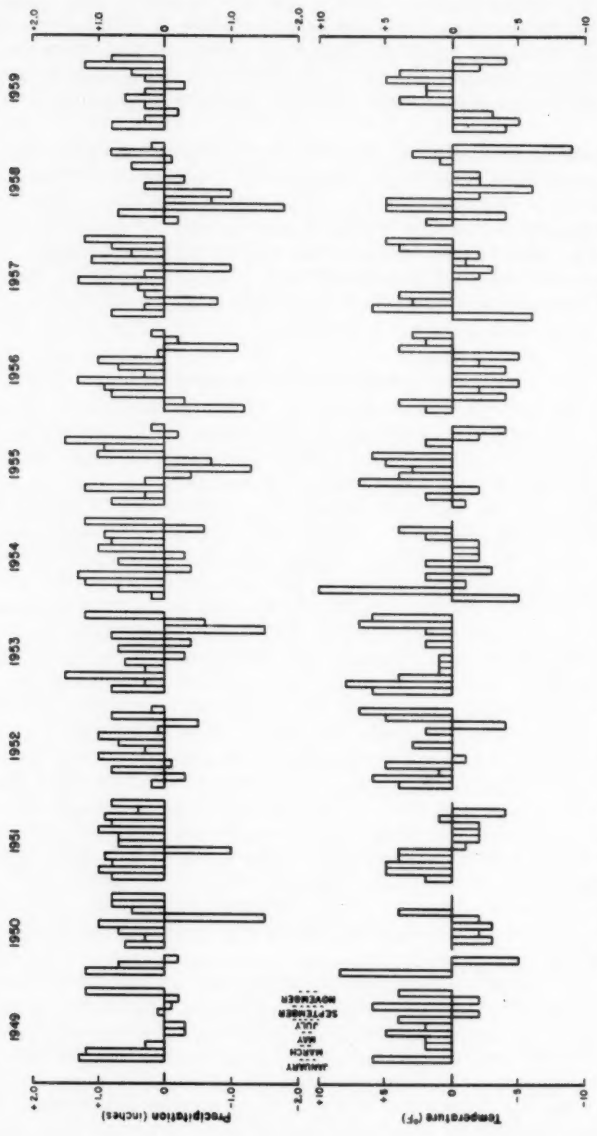


FIG. 7. Deviations from mean monthly precipitation and temperature for southcentral Ontario.

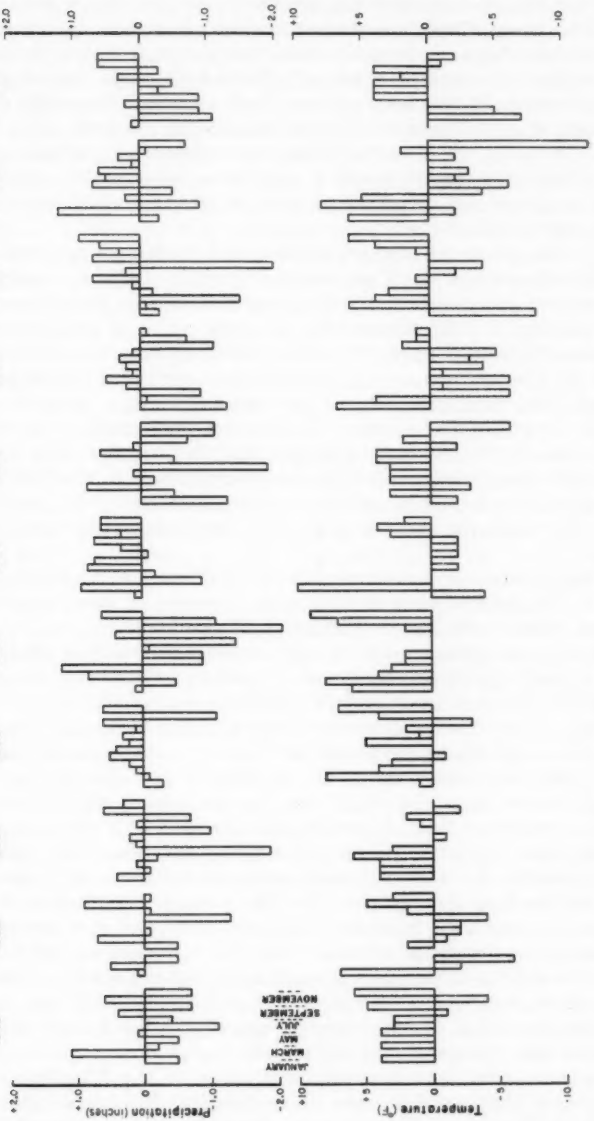


FIG. 8. Deviations from mean monthly precipitation and temperature for southern Quebec.

of the infected or invaded branches and stems, should partially determine the success of dieback. Invasions apparently do not usually proceed in proximal directions along either branches or leaders which are much larger than 1 in. in diameter. Also, when an invasion has proceeded along a branch to the bole, girdling is a common result only if the bole is less than 4 in. in diameter at that point. If the bole is larger, such an invasion usually results in the formation of a localized canker on the side of the bole (Fig. 5). This explains the observed size-class distributions of entirely or partially reddened trees. Only those trees in the smallest diameter classes are commonly killed by dieback in a single year and the proportion of a tree which may be killed in any one year is directly related to its size.

However, even when wounded branches and leaders of appropriate size become infected, dieback need not necessarily follow. On the contrary, the observations and experimental results presented in this paper support the belief that dieback usually occurs only when the vigor of the infected trees has been lowered. For example, it will be recalled that successful artificial production of dieback was accomplished only with trees which had been transplanted prior to inoculation. Of the naturally grown trees in the two lots which were inoculated without having been subjected to the shock inherent in transplantation, comparatively few became infected and none developed dieback. Although this apparent reduction in the efficiency of the fungi may be explained in several ways, relatively high host vigor is the one for which perhaps the greatest amount of attributive supporting evidence can be derived.

Most of the trees found affected during 1954 in either Ontario (20) or Quebec (15) had grown along roadsides or in exposed, open stands. Also, most of them were on shallow or excessively drained soils.

Furthermore, the influence of climate on the outbreak of dieback was recognized in both Provinces. In Ontario, Hord and Quirke (12) stated that the "extremely dry conditions" which prevailed during 1955 would account for the marked increase in incidence over that of the previous year. In Quebec, Pomerleau (15) evidently believed that the "unusually warm and dry weather" of 1952 and 1953 had caused the death, in 1954, of the affected trees, which were on unfavorable sites. No cankering was mentioned, although he noted that several saprophytic fungi (later (16, 17) reported as *Nectria* sp., *Dermea* sp., and *Valsa* spp.) had been found on needles, branches, and trunks of the dead trees. Clearly, the roles of climate and site had been so strikingly important in the death of Quebec trees that the possible significance of nominally saprophytic fungi was, understandably, not given primary consideration.

In an attempt to determine the extent to which readily available weather records can be shown to support general observations such as those that 1955 was a warm, dry year in Ontario and that 1952 and 1953 were similarly adverse in Quebec, Figs. 7 and 8 were prepared. Values for the deviations from normal mean temperatures and for the total precipitations for each month were calculated from the summary maps of the Monthly Weather Maps which are distributed by the Meteorological Branch of the Canada Department of Transport. Temperature deviations were plotted directly. Precipitation deviations were first determined by comparing the calculated

totals with long-term means. Averages of the mean monthly amounts (2) recorded at Sault Ste. Marie, Toronto, and Ottawa were arbitrarily selected to represent the designated part of south-central Ontario, while amounts from Montreal and Quebec City were averaged to represent the designated part of southern Quebec. Although monthly average deviations derived in this way will mask any very brief or very local extremes in temperature or rainfall, there is little reason to doubt that they reflect both the trends and the major variations through the given years.

From Fig. 7 it can be seen that, in southcentral Ontario, the growing season of 1949 was warm with essentially normal precipitation (warm: normal). Those of 1950 and 1951 were cool: wet, while that of 1952 was normal: wet. In 1953, the season was normal to slightly warm: normal to moist and became warm: dry late in the fall. On the average, 1954 was cool to normal: normal to wet, while the 1955 season was hot: dry. None of the succeeding summers appears to have been so unfavorable for tree growth and vigor, although the early spring of 1958 was hot: dry. In southern Quebec (Fig. 8), the summers of 1949, 1953, and 1955 appear to have been the least favorable. All three of these seasons tended to be much hotter and drier than normal.

It is interesting to note that an apparently similar outbreak of balsam fir dieback, which occurred in Minnesota and Wisconsin during the period 1933 through 1935 (5), coincided exactly with the "dust bowl" drought years, which were said to have been the worst ever recorded in eastern Nebraska, western Iowa, and Kansas (26). However, neither insects nor adverse climate were reported in connection with that outbreak. The relationship of the canker-producing fungus, *Cephalosporium album*, to the disease may have seemed so obvious that no other causal factors were sought.

The recent outbreak of balsam fir dieback in eastern Canada clearly seems to have been caused by a combination of factors acting jointly, even though it originally was attributed almost exclusively to fungal attack in Ontario and to adverse climate in Quebec. Probably the hot, dry weather which prevailed during 1953 was the factor which triggered the outbreak in both Provinces. However, even in Quebec the weather had not been sufficiently adverse to kill trees which were growing on good sites. Even if no organisms were involved there, which is unlikely, the joint action of climate and site was required. In Ontario, the influencing factors probably were climate, fungi, site, and insects. It is not implied that all of the several acting and interacting factors are of equal importance. The relative contribution of each to the dieback certainly varies from place to place and from year to year, but the basic principle of multiple causation evidently applies.

A parallel syndrome of multiple causation leading to similar cankering and dieback of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) apparently has existed in British Columbia (25, 13 et seq. ann., 18). In both the balsam fir and the Douglas fir diebacks, adverse climate apparently had acted to reduce the vigor of trees growing in situations which were marginal with respect to physiographic site, and the consequence was that nominally saprophytic fungi were able to attack them parasitically. This is consistent with the well-known concept of environmental predisposition of hosts to attack by "causal" organisms. However, the difference between the contribution

of the environment in these cases and in any instances where a more classical host–parasite relationship applies, probably is only a matter of degree. There may be an advantage, particularly in forest pathology, in giving greater consideration to the role of environment in diseases of all kinds than has been commonly done in the past.

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References

1. ANONYMOUS. 1940. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect Survey.
2. ANONYMOUS. 1948. Climatic summaries for selected meteorological stations in the Dominion of Canada. Vol. 1.
3. BALCH, R. E. 1952. Studies of the balsam woolly aphid, *Adelges piceae* (Ratz.) and its effects on balsam fir, *Abies balsamea* (L.) Mill. Can. Dept. Agr. Publ. No. 867.
4. BELYEA, R. M. 1952. Death and deterioration of balsam fir weakened by spruce budworm defoliation in Ontario. Part I. Notes on the seasonal history and habits of insects breeding in severely weakened and dead trees. Can. Entomologist, **84**, 325–335.
5. CHRISTENSEN, C. M. 1937. Cephalosporium canker of balsam fir. Phytopathology, **27**, 788–791.
6. CHRISTENSEN, C. M. and HODSON, A. C. 1954. Artificially induced senescence of forest trees. J. Forestry, **52**, 126–129.
7. DAVIDSON, A. G. 1954. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
8. DAVIDSON, A. G. and NEWELL, W. R. 1956. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
9. DAVIDSON, A. G. and NEWELL, W. R. 1958. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
10. FAULL, J. H. 1920–1921. *In* Report of the Minister of Lands and Forests of the Province of Ontario for 1920.
11. GRUENHAGEN, R. H., RIKER, A. J., and RICHARDS, C. A. 1947. Burn blight of jack and red pine following spittle insect attack. Phytopathology, **37**, 757–772.
12. HORD, H. H. V. and QUIRKE, D. A. 1955. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
13. MOLNAR, A. C. 1954. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
14. PEARCE, I. and SPAULDING, P. 1942. Plant pathology in relation to north-eastern forest wildlife cover. J. Wildlife Management, **6**, 194–202.
15. POMERLEAU, R. 1954. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rep. Forest Insect and Disease Survey.
16. POMERLEAU, R. and BENAZET, J. 1956. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
17. POMERLEAU, R. 1958. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
18. PORTER, W. A. 1957. Dieback of Douglas fir. Can. Dept. Agr., Forest Biol. Div., Bi-monthly Progr. Rept. **13**(5), 3.
19. QUIRKE, D. A. and HORD, H. H. V. 1954. *In* Can. Dept. Agr., Forest Biol. Div., Ann. Rept. Forest Insect and Disease Survey.
20. QUIRKE, D. A. and HORD, H. H. V. 1955. A canker and dieback of balsam fir in Ontario. Can. Dept. Agr., Forest Biol. Div., Bi-monthly Progr. Rept. **11**(6), 2.
21. RAYMOND, F. L. and REID, J. 1957. Comments on the agents responsible for the cankering and killing of balsam fir in eastern Canada. Can. Dept. Agr., Forest Biol. Div., Bi-monthly Progr. Rept. **13**(6), 1.
22. SEELER, E. V. 1940. A monographic study of the genus Thyronectria. J. Arnold Arboretum (Harvard Univ.), **21**, 429–460.

23. SWAINE, J. M. and CRAIGHEAD, F. C. 1924. *In* Studies on the spruce budworm (*Cacoecia fumiferana* Clem.). Part I. A general account of the outbreaks, injury and associated insects. Can. Dept. Agr. Tech. Bull. **37**, 3–26.
24. SWAN, H. S. D. 1954. New balsam fir disease. A note on a disease which is causing widespread balsam fir (*Abies balsamea*) mortality in eastern Canada. Pulp Paper Mag. Can. **55**(13), 249.
25. THOMAS, G. P. 1950. Two new outbreaks of *Phomopsis lokoyae* in British Columbia. Can. J. Research, C, **28**, 477–481.
26. WEAVER, J. E. and Anderson, F. W. 1936. Effects of the great drought on the prairies of Iowa, Nebraska, and Kansas. Ecology, **17**, 567–639.

STUDIES OF LIGNIN BIOSYNTHESIS USING ISOTOPIC CARBON

IX. TAXONOMIC DISTRIBUTION OF THE ABILITY TO UTILIZE TYROSINE IN LIGNIFICATION[1]

STEWART A. BROWN

Abstract

A survey of various plant species for the ability to utilize L-tyrosine as a lignin precursor has been extended, and the results obtained on a total of 21 species, representing seven families of monocotyledons and five of dicotyledons, are presented. Only grasses were able to convert L-tyrosine and L-phenylalanine to lignin with about equal efficiency, although two members of the Compositae and possibly one monocotyledon, *Triglochin maritima*, showed evidence of some ability to utilize tyrosine. One species of *Juncus*, *J. nodosus*, was unable to use phenylalanine efficiently and another, *J. balticus*, yielded conflicting results with regard to the utilization of both compounds. The findings are discussed in relation to known lignification pathways and taxonomic relationships.

Introduction

Results of previous research in this laboratory have suggested that the ability of plants to utilize L-tyrosine as a precursor of lignin is not widespread (1, 2). In fact it appeared to be restricted to the Gramineae, as the only two species found capable of using L-tyrosine as efficiently as L-phenylalanine (one of the best lignin precursors) were both grasses. In an attempt to explain this phenomenon C^{14}-labelled *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids were synthesized and fed to a grass and members of other families (3). The behavior of these compounds parallelled that of tyrosine. These findings were consistent with the theory that the two compounds are intermediates in the conversion of tyrosine to *p*-hydroxycinnamic acid, which is metabolized in all species thus far tested.

Although this route has not been ruled out, recent studies by Neish (10) have revealed the existence in grasses, but not in other species examined, of an enzyme, tyrase, which mediates the deamination of tyrosine to form *p*-hydroxycinnamic acid. Koukol and Conn (7) similarly have reported a phenylalanase in *Melilotus* catalyzing an analogous reaction of phenylalanine to cinnamic acid, which is known (9) to undergo hydroxylation to *p*-hydroxycinnamic acid. As the formation of this last compound is more simply explained by the reactions involving phenylalanase and tyrase than by the reaction sequence postulated earlier (3), it is now assumed that *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids are converted to lignin via tyrosine.

The present paper reports the results of a survey of a number of additional species which, with the results previously reported, was intended to provide a broader picture of the distribution of the ability to utilize tyrosine. It may also serve as a guide for future study of the relation between tyrase activity and tyrosine utilization for lignin synthesis.

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Experimental

All the species tested in the present work, as in previous studies, are either native to or extensively cultivated in the Saskatoon area. With the exception of *Triticum*, *Hordeum*, and *Fagopyrum*, which were greenhouse-grown, the plants used grew out-of-doors in soil. The native species were taken from their natural habitats. In most cases plants at or past the flowering stage were employed, as there is reason to believe that in herbaceous species the younger plants do not lignify at an appreciable rate.

Cut shoots were taken and in each case the ends were trimmed under water. They were then placed in the solutions described in Table I and kept either in a greenhouse or in a continuously illuminated growth chamber with a light intensity of at least 5000 lux (500 ft-c). In most cases uptake of the solutions was complete within 3 to 4 hours. *Triglochin maritima* and *Juncus balticus* (in the first of two experiments) required considerably longer periods, the former not absorbing its solution completely within 24 hours. In this case the uptake was calculated after C^{14}-analysis of the residual solutions. Another species, *Typha latifolia*, would not absorb any visible amount of the solutions, and could not be tested. At no time was any evidence of microbial growth seen in the solutions.

The plants were allowed to metabolize for approximately 24 hours after uptake of the solutions had ceased. They were then worked up as described previously (1), and the *m*-nitrobenzoylhydrazones of vanillin and syringaldehyde, obtained from the nitrobenzene oxidation of lignin, were combusted to carbon dioxide, which was analyzed for C^{14} using an ion chamber.

Results and Discussion

The results of the survey are presented in Table I. Species tested earlier are included here to facilitate comparison. Inasmuch as grasses, which are monocotyledons, were the only species utilizing tyrosine for lignification in the earlier investigations, the emphasis here has been placed on the monocotyledons in an effort to detect any extension of this metabolic peculiarity beyond the Gramineae. Representatives of seven families in this group have now been studied, as well as five families of dicotyledons. No gymnosperms have been examined, but the finding of Kratzl and Billek (8) that spruce will not convert *p*-hydroxyphenylpyruvic acid to lignin gives no reason to believe that tyrosine is a lignin precursor at a lower taxonomic level than the angiosperms. Although the present survey is of necessity very incomplete, an attempt has been made to run the gamut among both monocotyledons and dicotyledons from the Gramineae and Compositae down to the less highly developed families such as the Sparganiaceae and Salicaceae.

The efficiencies with which phenylalanine and tyrosine were utilized can be seen by a comparison of the dilution values in the last column on the right, the lower dilutions signifying more efficient utilization. It should be emphasized that the dilution figures are strictly comparable only in those cases where the precursors were fed at comparable dose rates; dilution values are sensitive to fluctuations in dose rates when these rates are low (12) as they were in almost all the species studied here.

The additional findings reported here reinforce the earlier conclusions that the ability to utilize tyrosine in lignification is very restricted among plants. With one doubtful exception, to be discussed later, the efficiency of tyrosine utilization approached or exceeded that of phenylalanine only among grasses. In most other species the efficiencies differed by a factor of at least 10, and in some cases more than 100. There is evidence that in the species of Compositae examined, especially *Zinnia*, tyrosine can undergo some conversion to lignin. Somewhat more efficient tyrosine utilization was also observed in *Triglochin*, a monocotyledon. In all these cases, however, the phenylalanine pathway is clearly the major one.

The genus *Juncus* appears to present some anomaly in the general picture. In *J. nodosus* the C^{14} of phenylalanine was diluted in the range 3150 to 4400, whereas in other species receiving comparable doses of phenylalanine (e.g. *Sparganium* and *Scirpus*) dilutions some 10 times lower were found. In *J. nodosus* tyrosine was utilized more poorly still. In contrast *J. balticus* appeared to have the poorest phenylalanine utilization of any plant yet tested, and it appeared to utilize tyrosine very well. As the results with *J. balticus* were so seriously out of line with those found for other members of the genus, a second experiment was done later in the season. This time the opposite result was obtained, with phenylalanine being used much more efficiently than tyrosine. The findings for *J. balticus* thus must be regarded as inconclusive. It should be pointed out, though, that in the first experiment with this species the administered phenylalanine was not readily translocated in the plant. Examination with the end-window probe of a monitor just before harvesting revealed a failure of the C^{14} fed as phenylalanine to be translocated more than 5 cm up from the cut end, or only about 1/10th of the total distance. The C^{14} from tyrosine was translocated normally. In the second experiment both compounds were readily translocated. These facts suggest that it is the data from the first experiment which should be regarded with suspicion.

At present the findings of this survey seem best explained by variations in the relative proportions of two enzymes, probably phenylalanase and tyrase, participating in alternative pathways through which prephenic acid is anabolized to *p*-hydroxycinnamic acid. In most species the tyrase activity is presumably very low, and little if any lignin is formed from tyrosine. In some species more activity is present, and a small amount of tyrosine can be utilized in lignification, but the major pathway still involves phenylalanine. Some members of the Compositae, which is regarded as the family of dicotyledons most highly developed morphologically, appear to fall in this category. The Gramineae, which occupy an analogous position among monocotyledons, seem to have developed a very active tyrase which enables the plant to utilize tyrosine on an equal basis with phenylalanine. The existence of other families or individual species with similar ability remains possible. It would be of considerable interest to examine the genus *Luzula*, also of the Juncaceae, which is similar in some morphological characteristics to the grasses. This genus is not native to the region of Saskatoon.

It is still uncertain whether the observed differences can be explained entirely on the basis of the relative tyrase and phenylalanase activities in various species. Correlation of enzyme activities with the ability of plants to

TABLE I

Utilization of L-phenylalanine and L-tyrosine in lignification by various species

Family	Subfamily	Species	Name	Compd. fed*		Compd. isolated (μc/mmole)		Dilution† (corr. for dry wt. of plant)	
				Specific activity (μc/mmole)	Dose (μmole/g dry wt.)	Vanillin	Syringaldehyde	Vanillin	Syringaldehyde
			1. Monocotyledons						
Sparganiaciae		*Sparganium multipedunculatum*	P	550	2.4	2.83	1.20	190	460
			T	1060	2.5	0.088	0.036	13,000	31,000
Scheucheriaceae		*Triglochin maritima*	P	747	2.4	0.646	0.537	1,200	1,400
			T	667	2.5	0.034	0.055	19,000	11,000
Alismaceae		*Alisma triviale*	P	550	3.4	2.71	1.65	200	330
			T	1060	3.2	0.182	0.098	5,600	11,000
Gramineae	Hordeae	*Triticum vulgare* var. Thatcher	P	723	15.3	0.65	1.6	1,100	450
			T	548	15.4	0.95	0.42	580	1,300
		Hordeum vulgare var. Montcalm	P	9560	0.12	1.44	1.62	6,600	5,900
			T	6620	0.16	1.55	1.62	4,100	3,900
	Festuceae	*Bromus inermis*	P	890	1.1	0.506	0.495	1,800	1,800
			T	980	1.1	0.608	0.650	1,600	1,500
	Agrostideae	*Calamagrostis inexpansa*	P	1400	0.41	0.855	1.06	1,640	1,320
			T	1360	0.35	0.454	0.664	2,600	1,780
Cyperaceae		*Carex laeviconica*	P	1400	0.35	0.376	0.636	3,700	2,200
			T	1360	0.32	0.005	0.004	240,000	300,000
		Scirpus validus	P	907	2.7	2.39	2.18	380	420
			T	845	2.2	0.052	0.053	13,000	13,000
Juncaceae		*Juncus bufonius*	P	907	3.0	2.42	2.30	380	390
			T	845	3.0	0.070	0.097	12,000	8,700

		Juncus nodosus	P	907	2.2	0.288	0.207	3,150	4,400
			T	845	2.2	0.041	0.038	21,000	22,000
		Juncus balticus	P	603	1.2	0.030	0.031	20,000	20,000
			T	616	1.3	0.665	0.599	1,020	1,130
			P	603	1.3	0.197	0.080	3,100	7,500
			T	616	1.3	0.009	0.003	60,000	200,000
Liliaceae		*Smilacina stellata*	P	1400	0.29	1.62	1.42	865	986
			T	1360	0.30	0.016	‡	88,000	—
			2. Dicotyledons						
Salicaceae		*Salix amygdaloides*	P	1400	0.26	0.789	1.46	1,780	959
			T	1360	0.29	0.011	0.031	130,000	49,000
		Populus balsamifera	P	2090	0.74	§	1.8	—	1,200
			T	1790	0.78	0.046	0.027	38,000	64,000
Polygonaceae		*Fagopyrum tataricum*	P	1400	0.27	1.44	1.18	972	1,190
			T	1360	0.35	0.005	0.006	360,000	300,000
Leguminoseae		*Caragana arborescens*	P	2090	0.88	4.7	1.8	445	1,200
			T	1790	1.22	0.054	0.025	46,000	100,000
		Melilotus officinalis	P	1400	0.24	0.865	0.950	1,620	1,470
			T	1360	0.26	0.010	0.010	160,000	160,000
Elaeagnaceae		*Elaeagnus commutata*	P	1400	0.27	0.741	0.595	1,890	2,360
			T	1360	0.28	0.008	0.005	170,000	280,000
Compositae	Helianthus	*Zinnia elegans*	P	734	2.3	2.24	1.69	328	434
			T	754	1.8	.187	0.482	3,100	1,210
		Lepachys columnifera	P	603	1.2	1.15	0.34	525	1,800
			T	616	1.5	0.08	0.08	10,000	10,000

*P = L-phenylalanine-U-C^{14}, T = L-tyrosine-U-C^{14}. The compounds were dissolved in 3 to 4 ml of distilled water and fed to plants with fresh weights of the order of 20 to 25 g.
†Observed dilution equals the specific activity of the administered compound divided by the values in columns 7 or 8.
‡Insufficient material for analysis.
§Sample lost.

utilize the respective substrates will be necessary before rigid conclusions can be drawn. It is hoped to pursue this question here at a later date.

The author wishes, finally, to draw attention to the possible taxonomic significance of this type of study. In the past decade or two, efforts have been made (4, 5, 6) to relate chemical constituents of plants to their classification. The work of Gibbs and his associates (4, 11) with regard to lignin composition is of special interest in the present context. The studies described here offer a direct and relatively simple means of demonstrating species differences in biochemical reactions, although such differences are naturally inferred in studies on components. Although a study of reactions leading to some other products may well be of value in classification, biochemical variations in respect to the formation of lignin could be of particular value because of the virtually universal distribution of this substance in families from the ferns up. As only 21 species have been tested in the present survey, the findings can hardly more than point up the possibilities of this approach. Any elaboration must be left to those whose interests are primarily in systematic botany.

Acknowledgments

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References

1. Brown, S. A. and Neish, A. C. 1955. Studies of lignin biosynthesis using isotopic carbon. IV. Formation from some aromatic monomers. Can. J. Biochem. and Physiol. **33**, 948–962.
2. Brown, S. A. and Neish, A. C. 1956. Studies of lignin biosynthesis using isotopic carbon. V. Comparative studies on different plant species. Can. J. Biochem. and Physiol. **34**, 769–778.
3. Brown, S. A., Wright, D., and Neish, A. C. 1959. Studies of lignin biosynthesis using isotopic carbon. VII. The role of *p*-hydroxyphenylpyruvic acid. Can. J. Biochem. and Physiol. **37**, 25–34.
4. Creighton, R. H. J., Gibbs, R. D., and Hibbert, H. 1944. Studies on lignin and related compounds. LXXV. Alkaline nitrobenzene oxidation of plant materials and application to taxonomic classification. J. Am. Chem. Soc. **66**, 32–37.
5. Erdtman, H. 1955. The chemistry of heartwood constituents of conifers and their taxonomic importance. Experientia, Suppl. No. 2, **14e**, 156–180.
6. Gibbs, R. D. 1945. Comparative chemistry as an aid to the solution of problems in systematic botany. Trans. Roy. Soc. Can. V, **39**, 71–103.
7. Koukol, J. and Conn, E. E. 1960. Abstracts. Pacific Slope Biochemical Conference, Davis, California, September, 1960.
8. Kratzl, K. and Billek, G. 1959. Über das Verhalten der 4-Hydroxyphenylbrenztraubensäure im verholzenden Gewebe der Fichte. Monatsh. Chem. **90**, 536–543.
9. McCalla, D. R. and Neish, A. C. 1959. Metabolism of phenylpropanoid compounds in Salvia II. Biosynthesis of phenolic cinnamic acids. Can. J. Biochem. and Physiol. **37**, 537–547.
10. Neish, A. C. 1960. Biosynthesis of *p*-coumaric acid in plants. Abstracts. Fifth Western Regional Conference, The Chemical Institute of Canada, Regina, Sask., September, 1960.
11. Towers, G. H. N. and Gibbs, R. D. 1953. Lignin chemistry and the taxonomy of higher plants. Nature, **172**, 25–26.
12. Watkin, J. E. and Neish, A. C. 1960. Biosynthesis of quercetin in buckwheat. Part III. Can. J. Biochem. and Physiol. **38**, 559–567.

STIMULATION BY LIGHT OF SPORULATION IN TRICHODERMA LIGNORUM (TODE) HARZ[1]

J. J. MILLER[2] AND JAMES REID[3]

Abstract

Exposure of growing cultures to white light caused subsequent formation of a ring of conidia-bearing mycelium at the location occupied by their advancing margins at the time of irradiation. Violet and blue light were very effective in stimulating sporulation, green was less so, and yellow and red were ineffective. An exposure to white light of the order of 1 foot-candle for 1 minute sufficed to initiate the sporulation response.

Introduction

In a study of the occurrence of yeasts in soils described by Miller and Webb (7), cultures of *Trichoderma* often developed in the nutrient medium of the dilution plates. In one such plate that had been left on the laboratory desk for several days conidial development was observed to be concentrated in concentric circles as in Fig. 1, indicating that some diurnal factor was influencing sporulation. This proved to be light, since the conidial formation by the fungus (identified as *T. lignorum* (Tode) Harz) in pure culture was found to occur in a few sparse tufts not annular in arrangement when grown in the dark. Brief exposure of a growing culture to light resulted in subsequent development of a ring of conidia-bearing mycelium at the location occupied by the advancing margin of the culture at the time of exposure. Cells a few millimeters back from the margin were evidently not stimulated to produce conidia even by quite strong light.

As this organism obviously provided favorable material for a study of the effect of light on conidial formation, several experiments were carried out to investigate the relationship between intensity and wavelength of light and conidial production.

Experimental

The nutrient medium used in all the experiments was potato dextrose agar (Difco), to which had been added 0.003% rose bengal dye. This had the advantage of slowing down the usually rapid growth of the fungus to one-third of the normal rate so that the rings were narrower and more sharply delimited. Petri dishes containing the medium were inoculated with mycelial transfers from a selected stock culture of *T. lignorum*. These cultures were then placed in a copper sterilizing can or else wrapped in opaque paper, and incubated at 27° C. When the cultures had grown to a suitable size, they were exposed in a photographic darkroom for varying lengths of time to light from a 25-watt

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incandescent lamp. Figure 2 demonstrates the response of a culture growing across a plate of this medium to exposure to light for 1 hour on successive days, and for comparison a plate exposed to continuous light is shown in Fig. 3.

To determine the amount of light required to produce a ring of conidia-bearing mycelium, cultures were exposed at varying distances from the light source. The light intensity was estimated with a General Electric light meter, model 8DW40Y2. When exposure time was sufficiently long to permit, the margin of the colony was marked on the underside of the petri dish with a glass-marking pencil.

TABLE I

Effect of exposure to light on sporulation in *Trichoderma*

Experiment No.	Light intensity (ft-c)	Maximum exposure that did not form rings (seconds)	Minimum exposure required to form rings (seconds)
6	1	60	120
7	1	—	16–30
4	2	5–10	10–15
5	2	5–10	10–15
3	2	24	26
2	2–2.5	—	15
10	3	4	8
9	4	4	8
8	5	0–2	4
1	30	0	1

The results of 10 such experiments are given in Table I. At a light intensity of 2 foot-candles at least 10 seconds' exposure was required for subsequent ring formation. Not all experiments, however, gave identical results: for instance, in experiment 3 at a light intensity of 2 ft-c at least 26 seconds was required. Nevertheless, the values obtained indicate that a small amount of white light, of the order of 1 ft-c for 1 minute, sufficed to initiate sporulation. The location of the ring of conidia-bearing mycelium appeared to coincide with the margin of the colony at the time of exposure, confirming the observation mentioned above that only the young cells responded to light stimulation.

The effectiveness of light of different wavelengths was compared by exposing growing cultures to light from a mercury lamp using filters that allowed passage of certain wavelengths only.

The results of these experiments are given in Table II. As the light meter used was about 70% as sensitive in the blue-green as in the green-yellow, the incident light energy must have been somewhat greater at the former wavelengths than that stated in Table II. Nevertheless, the data provide evidence that the violet and blue wavelengths were very effective in inducing conidial formation, the green less so, and the yellow ineffective. The latter point was supported by further experiments in which growing cultures were exposed to a Westinghouse yellow incandescent light bulb (27 ft-c for 10 minutes) and to a Wratten series OA yellow photographic filter (2 ft-c for 60 minutes). No rings formed in either case. A red incandescent light bulb (4 ft-c for 60 seconds) gave the same result.

PLATE I

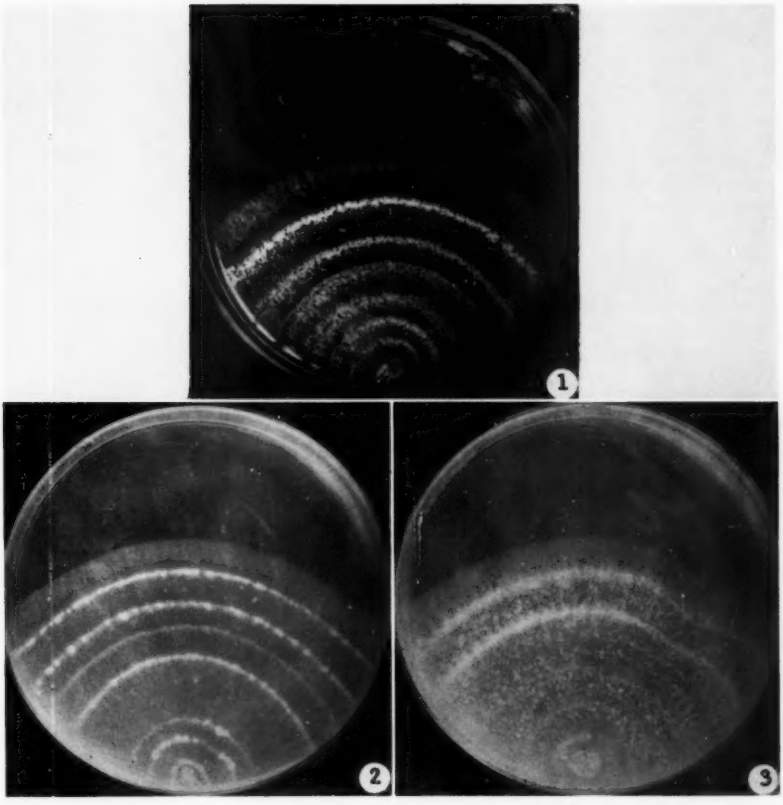

FIG. 1. Response of growing culture of *T. lignorum* to diurnal fluctuations in light from a north window.

FIG. 2. Response of *T. lignorum* to exposure to light from a 25-watt incandescent lamp for 1 hour on successive days. The wide space between the fourth and fifth rings from the top represents a period of 48 hours during which no exposure was made.

FIG. 3. Response of *T. lignorum* to exposure on laboratory bench to continuous light from a 25-watt incandescent lamp plus diurnal fluctuations in light from north window.

TABLE II

Effect of light of different wavelengths on sporulation in *Trichoderma*

Wavelength	Intensity (ft-c)	Time of exposure (sec)	Conidial development
Violet (4047, 4078 Å)	1	20	Marked ring
Blue (4358 Å)	10	10	Marked ring
Green (5461 Å)	2	20	Slight ring
Yellow (5791, 5769 Å)	10	10	No ring

In these experiments no assessment was made of the light absorbed by mycelium and medium, and transformed into heat. It seems unlikely, however, that light of such low intensity and duration would produce enough heat to be of significance.

Discussion

Gutter (3), in a study of several isolates of *Trichoderma viride*, has obtained results similar in many respects to those reported here. Exposure to light of 85–90 lux intensity for 20–60 seconds was sufficient to initiate sporulation. The writers' finding that exposure to light of 1 ft-c intensity for 1 minute induced sporulation of *T. lignorum* indicates that a low but similar amount of light suffices to induce sporulation in representatives of both species. Gutter found that sporulation of most of his strains was induced only in that part of the mycelium which was at a distance of about 5–10 mm from the advancing hyphal tips at the time of irradiation. The writers did not observe this effect. The rings of conidia appeared always to form at the location of the advancing edge, a response which, incidentally, was also shown by Gutter's isolate No. 12.

Red light was found by Gutter to be ineffective in inducing sporulation of *T. viride*. The writers also observed this and showed yellow light to be similar. Barnett and Lilly (1) working with several fungi, including an isolate of *T. lignorum*, observed stimulation of sporulation by white light. Blue light gave an effect similar to white, whereas in red light sporulation was reduced, greatly delayed, or absent. Similar results have been reported for a number of other fungi (2, 6, 5). When fungi need light to sporulate, it is nearly always the blue end of the visible spectrum that is the more effective. (See Hawker (4).)

As Hawker has observed, many fungi either in natural habitat or in artificial culture produce alternate zones of different growth as a result of diurnal fluctuations in light intensity. Most of these result from stimulation (or occasionally from inhibition) of sporulation by light. The results of the present study provide another illustration of this general response. As the organism studied is presumably soil-dwelling, the ecological value of producing spores specialized for aerial dissemination only under the influence of light seems evident.

Acknowledgment

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References

1. BARNETT, H. L. and LILLY, V. G. 1952. The effect of colour of light on sporulation of certain fungi. Proc. West Va. Acad. Sci. **24**, 60–64.
2. FULKERSON, J. F. 1955. The relation of light to the production of pycnidia by *Physalospora obtusa*. Phytopathol. **45**, 22–25.
3. GUTTER, Y. 1957. Effect of light on sporulation of *Trichoderma viride* Pers. ex Fr. Bull. Research Council Israel, Sect. D, **5**(4), 273–286.
4. HAWKER, L. E. 1957. The physiology of reproduction in fungi. Cambridge University Press, London. pp. 1–128.
5. ISAAC, I. and ABRAHAM, G. H. 1959. Saltation and zonation formation in *Verticillium lateritium*. Can. J. Botany, **37**, 801–814.
6. MCLELLAN, W. D., BORTHWICK, H. A., BJORNSSON, I., and MARSHALL, B. H. 1955. Some responses of fungi to light. Phytopathol. **45**, 465.
7. MILLER, J. J. and WEBB, N. S. 1954. Isolation of yeasts from soil with the aid of acid, rose bengal, and oxgall. Soil Sci. **77**, 197–204.

INCORPORATION OF P^{32} INTO RIBONUCLEIC ACID OF RUSTED WHEAT LEAVES[1]

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Abstract

P^{32}-labelled orthophosphate was fed to rusted and non-rusted wheat leaves. The ribonucleic acid (RNA) isolated from this material was purified and its specific activity determined. The specific activity of RNA from *susceptible* reacting tissue had increased 60–208% 4½ days after inoculation, but 7½ days after inoculation it was not markedly different from RNA preparations of uninoculated leaf material, indicating an increase and eventual decline in RNA synthesis of the host–parasite complex. In the *resistant* reaction incorporation of P^{32} into the RNA was unaltered by infection. The possible metabolic significance of these observations is discussed.

Introduction

Metabolic investigations involving obligate parasites have demonstrated the possible significance of phosphorus-containing compounds in these host–parasite relations (7, 11). When introduced into detached leaves a number of labelled compounds, including P^{32}-labelled orthophosphate, are selectively accumulated near sites of infections (1, 2, 3, 9, 10, 13). No information is available on the extent to which the introduced orthophosphate is metabolized and in which chemical form this label is eventually contained in parasitized host tissues. One important aspect of the phosphorus metabolism of rusted leaves may be the synthesis of nucleic acids by the host and (or) the parasite as suggested by the increased RNA stainability of host nuclei after infection and the high RNA content of hyphal tips of the rust fungus (6). In the present paper results are reported on the isolation, purification, and specific activity of RNA from resistant and susceptible reacting wheat leaves prior to and following infection with rust.

Materials and Methods

Six-day-old wheat seedlings of varieties Little Club and Khapli, grown in growth chambers at 28° C, were inoculated with physiological race 21 of *Puccinia graminis tritici* or with physiological race 52*a* of *Puccinia recondita*, the former giving rise to a susceptible reaction on Little Club and a resistant reaction on Khapli, the latter producing susceptible reactions on both host varieties. The inoculated plants remained in moist chambers for 36 hours at 20° C and were subsequently transferred into growth chambers at 28° C. Uninoculated control plants were treated in the same manner. The first leaves were detached 4½ days and 7½ days after inoculation, put with their cut ends into tap water or Knop's nutrient solution for 2 hours, transferred to similar solutions containing P^{32}-labelled orthophosphate (1.5 to 3.5 mc/ml),

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and maintained for 8 hours at 27° C, 55% relative humidity, and approximately 8000 lux (834 ft-c). After exposure to the radioactive solutions, samples of the leaf material were retained for determining dry weight (5 to 10 mg) and the radioactivity taken up (0.02 to 0.15 mc/mg dry weight). Sample leaves were cut into small pieces, immediately plunged into liquid air, and ground to a fine powder with solid CO_2. After sublimation of the CO_2 at $-5°$ C each sample was extracted with 150 ml boiling 96% ethanol and the resulting suspension filtered through sintered glass (G-4, Schott and Gen., Jena). The residue was washed several times with acetone to remove chlorophyll.

For extraction of RNA from the air-dried powder, the method of Markham (4) was applied. However, in order to obtain sufficiently pure RNA preparations, it was necessary to repeat the ethanol/acetic acid and HCl-precipitation steps. The purified RNA preparations were taken up in 10 ml 0.5 *M* NaCl solution (pH 7.5).

Phosphorus determinations were carried out in test tubes according to Umbreit *et al.* (p. 273) (12). For spectrophotometric analysis of RNA preparations a Zeiss spectrophotometer (model PMQ II) with 1-cm quartz cells was used. For determination of activity the RNA solutions were diluted 11-fold and the activity measured with a beaker-shaped G-M tube accommodating liquids. All counts per minute recorded were corrected to give the activity at time of harvesting.

Results and Discussion

Accurate and reliable determinations of specific activity depend on the purity of the compound under investigation. Dialyzed solutions of RNA preparations (in 5×10^{-2} *M* NaCl, at pH 6.0; 1.86 mg phosphorus/ml) exhibited absorption minima at 234 to 237 mμ and absorption maxima at 258 to 259 mμ. In all cases extinction at 320 mμ was negligible and the ratio E_{max}/E_{min} very close to 1.8, both characteristics pertaining to RNA solutions of high purity (4). The absorption at 258 mμ calculated for 1 *M* RNA-phosphorus ranged from 7000 to 8630, indicating that most of the RNA had remained undegraded during extraction and purification (4).

Enzymatic degradation with ribonuclease under standard conditions (5) yielded equal amounts of breakdown products when the isolated RNA preparations were compared with a commercially available RNA sample (Schuchardt, "reinst"). Possible contaminations with protein were below limits of detection.

Since the specific activity of RNA may depend in part on the isotope concentration in the tissue, an effort was made to determine the total activity taken up per mg dry weight or phosphorus content of leaf tissue. This information was later used to correct the experimentally determined specific activity of RNA. Variations in the amount of activity taken up by individual samples (Table I) are primarily caused by differences in the activity of isotope solutions in various experiments and by different rates of water uptake and transpiration rates in healthy and inoculated leaves. In spite of rigidly controlled and standardized conditions during exposure to the isotope solution,

TABLE I

P^{32} taken up by noninoculated and rusted wheat leaves and specific activity of RNA preparations isolated from this material

Expt. No.*	Sample	Days after inoculation	Rust reaction of host tissue	Activity taken up by leaf material: c/m/mg dry wt. $\times 10^3$	Activity taken up by leaf material: c/m/mg total P $\times 10^5$	Specific activity of isolated RNA: Exptl. values (c/m/mg RNA P $\times 10^4$)	Specific activity of isolated RNA: Corrected for activity taken up† (c/m/mg RNA P $\times 10^4$)	Specific activity of isolated RNA: Corrected for activity taken up† (noninoculated control = 100)
			Inoculum: *P. recondita*					
I	Little Club, healthy	4½	—	7.2	6.9	12.4	12.4	100
	Little Club, rusted	4½	Susceptible	8.0	6.6	21.0	21.9	176
	Khapli, healthy	4½	—	7.1	7.1	11.8	11.8	100
	Khapli, rusted	4½	Susceptible	7.7	7.3	18.9	19.5	165
			Inoculum: *P. graminis tritici*					
II	Little Club, healthy	4½	—	1.7	1.5	1.7	1.7	100
	Little Club, rusted	4½	Susceptible	0.75	0.68	2.3	5.2	308
III	Little Club, healthy	4½	—	5.9	5.1	6.4	6.4	100
	Little Club, rusted	4½	Susceptible	3.9	2.8	8.6	15.7	245
IV	Little Club, healthy	4½	—	12.6	11.5	10.7	10.7	100
	Little Club, rusted	4½	Susceptible	6.0	4.5	11.4	29.3	274
	Khapli, healthy	4½	—	12.0	11.1	9.3	9.3	100
	Khapli, rusted	4½	Resistant	12.3	12.2	10.4	9.5	102
V	Little Club, healthy	7½	—	22.6	17.7	11.3	11.3	100
	Little Club, rusted	7½	Susceptible	27.0	20.1	10.5	9.2	81
	Khapli, healthy	7½	—	25.8	25.7	12.3	12.3	100
	Khapli, rusted	7½	Resistant	20.3	19.1	11.3	15.2	124

*Isotope solutions were prepared with tap water in expts. I and II, with Knop's nutrient solution in expts. III, IV, andV. The activity contained in these solutions was 3.3 μc/ml (expts. I, IV, and V), 1.5 μc/ml in expt. II, 3.5 μc/ml in expt. III.
†Based on activity taken up per mg total P of leaf material (see column 6 of this table).

replicate samples showed considerable variation in the amount of activity taken up by each individual leaf. This is believed to be due to partial and uncontrollable blockage of xylem vessels on detachment of leaves. Up to five leaves of each sample were therefore used to determine the average activity taken up.

The various RNA preparations exhibited activities between 0.56 and 12.4 $\times 10^3$ c/m/ml; with a counting efficiency of 9.8% of the equipment used, this corresponds to approximately 0.08 to 1.0 mc/mg RNA phosphorus. An account of the relative specific activities for RNA preparations, as determined by analyses and corrected for uptake of radioactivity into the leaf material, is given in Table I. Corrections for isotope uptake, whether based on dry weight or total phosphorus, did not substantially alter the trend observed for incorporation rates of P^{32} into the RNA of the various samples.

The increase in specific activity of the RNA during the first stages of disease development ($4\frac{1}{2}$ days after inoculation) in the *susceptible* reaction can be attributed to an increased biosynthesis of RNA in the host–parasite complex. At $7\frac{1}{2}$ days after inoculation the specific activity of the RNA isolated from inoculated tissue is not markedly different from that of uninoculated leaf material, indicating a decline of RNA synthesis after the initial period of spore formation of the parasite. This view is supported by the recent report (8) that the RNA content of Little Club leaves is nearly doubled 7–9 days after inoculation with rust, but may decline again as the infections age. The observed differences in specific activity may, alternatively, reflect a postinfectional change in pool sizes of immediate RNA precursors. Further studies, employing microautoradiographic techniques, are in progress which may clarify whether the early increase and eventual decline in incorporation occur chiefly in the affected host tissue and (or) in the developing fungus.

In the *resistant* reaction the incorporation of P^{32} into the RNA of the host–parasite complex is essentially unaltered by infection. This may be due to the sparse growth of fungal mycelium in these tissues and (or) the failure of the affected host tissue to respond with increased synthesis of RNA. As with most biochemical analyses of parasitized tissue, it is difficult to discriminate between the metabolic activity of the obligate pathogen and the host. The chemical composition of RNA from rusted wheat leaves differs only slightly from that of healthy leaves (8). An interpretation of the metabolic significance of the above observations must therefore be purely speculative, until information is available on the histological localization of the incorporation sites.

The relatively low specific activity of RNA from leaf rust infected tissue $4\frac{1}{2}$ days after inoculation can be attributed to the fact that *P. recondita* completes its cycle sooner than *P. graminis tritici* and may at this time already experience a decline in incorporation of P^{32} into the RNA, which for stem rust infected leaves was observed as late as $7\frac{1}{2}$ days after inoculation.

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References

1. BALDACCI, E., BETTO, E., FOA, R., e VOLPI, A. 1957. La richiesta di fosforo nei tessuti di piante invasi da funghi o da virus, esaminata con l'impiego des P^{32}. Ricerca sci. **27**, 2674–2676.
2. BETTO, E., FOA, R., e VOLPI, A. 1958. La competizione per il fosforo fra Uromyces appendiculatus (Pers.) Link e Phaseolus vulgaris L. studiata con P^{32}. Phytopathol. Z. **32**, 283–292.
3. GOTTLIEB, D. and GARNER, J. M. 1946. Rust and phosphorus distribution in wheat leaves. Phytopathology, **36**, 557.
4. MARKHAM, R. 1955. *In* Modern methods in plant analysis. *Edited by* K. PAECH and M. V. TRACEY. Springer-Verlag, Berlin.
5. MCDONALD, M. R. 1955. *In* Methods in enzymology. *Edited by* S. P. COLOWICK and N. O. KAPLAN. Academic Press, Inc., New York.
6. PERSON, C. O. 1960. A preliminary note on the histochemical localization of DNA and RNA in rust-infected wheat leaves. Can. J. Genet. and Cytol. **2**, 103–104.
7. POZSÁR, B. I. and KIRÁLY, Z. 1958. Effect of rust infection on oxidative phosphorylation of wheat leaves. Nature (London), **182**, 1686–1687.
8. QUICK, W. 1960. Ribonucleic acid relationships in wheat leaves infected with the stem rust fungus. Unpublished M.A. Thesis, University of Saskatchewan, Saskatoon, Sask.
9. SHAW, M., BROWN, S. A., and RUDD JONES, D. 1954. Uptake of radioactive carbon and phosphorus by parasitized leaves. Nature (London), **173**, 768.
10. SHAW, M. and SAMBORSKI, D. J. 1956. The physiology of host–parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Can. J. Botany, **34**, 389–405.
11. TURIAN, G. 1957. Exaltation de l'activité phosphatasique dans le latex d'Euphorbia verrucosa L. parasitée par Uromyces scutellatus (Schr.) Lév. ses relations avec le métabolisme auxinique. Phytopathol. Z. **28**, 275–280.
12. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. 1957. Manometric techniques. Burgess Publishing Co., Minneapolis, Minn.
13. YARWOOD, E. C. and JACOBSON, L. 1955. Accumulation of chemicals in diseased areas of leaves. Phytopathology, **45**, 43–48.

NATURAL SELECTION AMONG HYBRIDS BETWEEN CARDUUS ACANTHOIDES AND C. NUTANS IN ONTARIO[1]

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Abstract

Natural hybridization is occurring between two introduced thistles, *Carduus acanthoides* L. and *C. nutans* L., in the southern half of Grey County, Ontario. The morphological variation of plants in 37 fields, including a field near Priceville previously studied in 1952, was evaluated by means of a hybrid index in 1957. *Carduus nutans* was first established in the hybrid area and *C. acanthoides* was introduced later, probably from the region northwest of Owen Sound, into waste land near Flesherton, where hybridization first occurred. Many large hybrid populations were found in the southern portion of Grey County and most of these hybrids were very similar to *C. acanthoides* in morphology. It was concluded that there is a numerical supremacy of *acanthoides*-type hybrid segregates in the hybrid area, and that such hybrids are better adapted for survival under local conditions than *C. nutans* and will probably supplant the latter species.

Introduction

Natural hybridization between two species of introduced thistles, *Carduus acanthoides* L. var. *acanthoides* and *C. nutans* L. var. *nutans*, was first noted in 1951 in Grey County, Ontario. In the following year the population of one field containing both parental species and hybrids was studied and the general extent of hybridization in the county was reported by Moore and Mulligan (3). The morphological variation of hybrid plants was evaluated by means of a hybrid index score. The species differ in chromosome number *C. acanthoides* $2n=22$, *C. nutans* $2n=16$; among the hybrids all intervening chromosome numbers were found. There was a close correlation between chromosome number and hybrid index; hybrids with the chromosome number of a first generation hybrid ($2n=19$) had an index intermediate between those of the parental species. Plants with other aneuploid numbers had a phenotype in keeping with their chromosome number. Many plants were later raised from seed collected from hybrid plants and it was noted that seedlings of somatic number 22 were much more abundant than those of other numbers. This fact suggested that ecological and gametic selection may be acting in this area to favor production of plants with the chromosome number and morphology of *C. acanthoides*.

Grey County was revisited in July of 1957 for the purpose of learning what change in the relative abundance the parental species and the hybrids may have undergone in the preceding 5 years. It was the intention also to observe whether changes in index balance had occurred, whether hybrid populations are segregating toward the phenotype of either parent, and to observe what success the parental species and hybrids may be having in com-

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petition for the available habitats. The observations of the 1952 and 1957 surveys will provide historical information for future comparison.

Materials and Methods

The hybrid index developed in the 1952 survey was again used in the present study. Plants were scored on the bases of the following six characters.

Head solitary	2	Heads clustered	0
Heads nodding	4	Heads erect	0
Peduncles not spiny-winged	4	Peduncles spiny-winged	0
Phyllaries reflexed	4	Phyllaries spreading	0
Phyllaries unmarked	1	Phyllaries marked	0
Phyllaries contracted at the base	4	Phyllaries not contracted	0

The sum of the score of each character of one plant is the hybrid index of the plant. When scored in this manner, typical *C. acanthoides* has an index of 0 and typical *C. nutans* has an index of 19 points and hybrids would have intermediate values. The characters which separate these *Carduus* species are described in detail by Mulligan and Frankton (2).

Approximately 150 miles of road, chiefly along Provincial Highways 4, 6, and 10 were travelled and a careful watch for *Carduus* plants in the adjacent fields was maintained. The parental species could be recognized from the road. In the area in which hybrids occurred, stops were made at intervals of approximately 1 mile and the nearest field containing *Carduus* plants was entered and the population studied in detail. These fields, called hereafter "index areas" were assigned numbers from 1 to 36, as shown on the map (Fig. 1). The nature of the population, whether purely *C. acanthoides* or *C. nutans* or a mixture of hybrids with or without parents, is indicated by the symbol shown with the area number. Numerous other fields were observed without close inspection and the nature of the population of these fields is shown by unnumbered symbols.

When the fields examined contained a large and variable population the following procedure was used. The whole area was inspected to determine whether hybrids alone were present or whether plants typical of either or both parental species occurred. The abundance and location of plants of different types were roughly mapped and then plants selected at random from each type were scored by the hybrid index in the field. So far as the local situation permitted, plants representative of different parts of the field and of different phenotypes were indexed.

The following summaries show the location, type of habitat, and present nature of the *Carduus* population.

Observations

The soil types of the areas surveyed have been classified by Gillespie and Richards (1) as follows: Osprey loam—index areas 1, 2, 27, 28, and 36; Donnybrook sandy loam—index areas 3, 6, 7, 10, 11, 12, 13, and 31; Pike Lake loam—index areas 4, 5, 8, 9, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 29, and 35; Harriston loam—index areas 24, 26, 30, 32, 33, and 34. All of these soils are well drained, moderately to very stony, often hilly, and very susceptible to water erosion. Most of the index areas were pastures or, occasionally,

abandoned farm land, and in many fields there was little competition from other plants.

Priceville Field

This area, previously studied by Moore and Mulligan (3) in 1952, is located 1 mile east of Priceville in Conc. IN, Artemesia Township. The study area, about 1700 sq. ft, was isolated at the top of a hill at an elevation of 1600 ft above sea level and about 50–75 ft above the nearby road. The grass cover at the time of the earlier survey was poor, and the pasture was not being grazed, but in 1957 the grass cover was fairly good and cattle were grazing at this location. *Carduus* was relatively common here in 1952, but in 1957, because of increased competition from other plants, the *Carduus* population was substantially reduced. In 1952 both parental species were present in small amounts and also many hybrids that were of low index score. In the sample of 26 plants indexed in 1952, 17 plants scored from 1 to 9 (av. 5.3), 5 had indices from 10 to 16 (av. 12.2), 2 had the index of *C. acanthoides* (0), and 2 that of *C. nutans* (19). In 1957 all the plants present on this hill were hybrids, and these had segregated even closer towards *C. acanthoides* than those seen in 1952. All 24 plants in flower on top of this hill in 1957 were indexed, 22 had indices from 1 to 9 (av. 3.6), and 2 plants had indices of 11 and 16. Both parents had been eliminated from this area since 1952. The fact that only hybrids, most of which had a low index score, occurred in this field in 1957, although both parental species and a wide range of hybrids are known to have been present 5 years before, indicates that *acanthoides*-type hybrids have a survival value superior to that of both species. It is proof also that a field presently occupied solely by hybrids has not necessarily been colonized by hybrids but may once have contained both species and been a center of hybridization.

Index Area 1

Location: 2½ miles northwest of Owen Sound, on road to Kemble. Three very stony, moderately sloping pastures were occupied by a large stand of *Carduus* plants, all typical *C. acanthoides*.

Index Area 2

Location: 2 miles north of Chatsworth, Conc. I, lot 1, Sullivan Twp. A large stand of plants was growing in several very stony, moderately sloping pastures, all plants were typical *C. acanthoides*.

Index Area 3

Location: 3.8 miles south of Chatsworth, Conc. I, lot 13, Sullivan Twp. Scattered plants of *Carduus* in a rocky pasture, all were typical *C. nutans*.

Index Area 4

Location: 3 miles south of Williamsford, Conc. I, lot 63, Holland Twp. A dense stand of *Carduus* occupied a rocky, rolling pasture, all plants were typical *C. acanthoides*.

Index Area 5

Location: 2.2 miles south of Doronock, Conc. I, lot 13, Glenelg Twp. All *Carduus* plants in this rocky, rolling pasture were typical *C. nutans*.

Index Area 6

Location: 1 mile south of Durham, Conc. I, lot 28, Glenelg Twp. *Carduus* plants occupied a pasture with a central, high, dome-shaped hill. A large patch of *C. nutans* covered one side of the hill and on top of the hill, about 40 yards from the *C. nutans* stand, was a small patch of *C. acanthoides*. Between the two patches were occasional hybrids with low indices. Of 20 plants indexed, 3 were hybrids with indices from 2 to 6 (average 4.0), 5 were typical *C. acanthoides*, and 12 were typical *C. nutans*.

Index Area 7

Location: 2.6 miles south of Durham, Conc. I, lot 6, Egremont Twp. The *Carduus* plants circled a lone spruce tree and extended from this ring across the pasture at the end of the field remote from the highway. Mostly hybrids, few *C. acanthoides*, and no *C. nutans* occurred here. Sixteen plants were indexed; 14 were hybrids with indices from 1 to 6 (av. 4.5) and 2 had an index of 0.

Index Area 8

Location: 6.7 miles south of Durham, Conc. I, lot 25, Egremont Twp. Plants were growing within and about the remains of a foundation of an old house in a pasture. All the *Carduus* plants appeared to be hybrids that had segregated strongly towards *C. acanthoides*. The 20 plants indexed were all hybrids with indices from 1 to 6 (av. 4.4).

Index Area 9

Location: 6.8 miles south southeast of Durham, Conc. II, lots 30 and 31, Egremont Twp. A pasture with a central high area was occupied chiefly by hybrids, but also a few *C. acanthoides* and *C. nutans*. Of 24 plants indexed, 15 were hybrids with indices from 2 to 9 (av. 3.0), 3 were hybrids with indices from 13 to 16 (av. 14.7), 4 plants had the hybrid index of *C. acanthoides* (0), and 2 plants had an index of 19.

Index Area 10

Location: $6\frac{3}{4}$ miles west of Hopeville, Conc. XVI, lot 12, Egremont Twp. A dense stand of *Carduus*, mostly *C. acanthoides*, few hybrids, and no *C. nutans*, was seen in a gravel pit at this location. Fifteen plants were indexed; six were hybrids with indices from 1 to 4 (av. 3.3) and nine plants had the hybrid index of *C. acanthoides* (0). The proportion of hybrids in the sample indexed is much larger than the proportion of hybrids in the population.

Index Area 11

Location: 5 miles west of Durham, Conc. IN, lot 21, Bentinck Twp. This large rolling pasture, and other pastures on the adjacent hills and valleys, were covered with dense stands of *acanthoides*-type hybrid segregates and relatively few plants of apparently typical *C. acanthoides*. There was a small patch of hybrids resembling *C. nutans*-type by the road. The owner stated that it was only recently that these thistles have reached such great abundance in his fields. Of 31 plants indexed, 19 were hybrids with indices from 1 to 4 (av. 2.1), 6 were hybrids with indices from 7 to 15 (av. 10.8), and 6 plants had an index of 0. The six plants with indices ranging from 7 to 15 were growing in the small patch of *nutans*-type hybrids by the road.

Index Area 12

Location: 2.5 miles west of Durham, Conc. IN, lot 31, Bentinck Twp. This field was one of the best pastures seen in the hybrid area and the 20 plants indexed represented almost the entire *Carduus* population. Seven plants were hybrid with indices ranging from 1 to 4 (av. 2.3), two were hybrids with indices of 11 and 17, and the remaining 11 plants were typical *C. nutans*.

Index Area 13

Location: 1 mile west of Durham, Conc. IS, lot 37, Bentinck Twp. Plants growing around an old house foundation were mostly *C. nutans* but there were also some hybrids with indices close to *C. acanthoides*. Twenty plants were indexed; six were hybrids, indices from 2 to 4 (av. 3.3), and 14 were typical *C. nutans* with an index of 19.

Index Area 14

Location: 3 miles east of Durham, Conc. IN, lot 5, Glenelg Twp. All *Carduus* plants in this pasture were *C. nutans* with an index of 19.

Index Area 15

Location: 4½ miles east of Durham, Conc. IN, lot 11, Glenelg Twp. *Carduus* plants in this pasture were mostly hybrid with a complete range of index scores from 1 to 17; a few plants of *C. acanthoides* and *C. nutans* also occurred. Of 15 plants indexed, 8 were hybrid, indices 1–9 (av. 6.0), 4 had indices from 10 to 17 (av. 13.0), 2 had an index 0, and 1 had an index of 19.

Index Area 16

Location: 5½ miles east of Durham, Conc. IS, lot 22, Glenelg Twp. Plants were growing in a level pasture and parts of an adjacent grainfield. The grain had been drilled into a pasture and *Carduus* was growing where the stand of grain was very sparse and in a strip that lacked grain. Mostly *C. nutans*, no *C. acanthoides*, and some hybrids tending towards *C. acanthoides* occurred in these fields. Twenty-four plants were indexed; nine were hybrids, indices 2–9 (av. 4.1), and the remaining plants were *C. nutans* with a hybrid index of 19.

Index Area 17

Location: 6.8 miles east of Durham, Conc. IS, lot 32, Glenelg Twp. The *Carduus* plants growing in gravelly waste land at this location were mostly *C. nutans*, with a small patch of *nutans*-type segregates towards the back of the field. Of 20 plants indexed, 1 hybrid had an index of 6, 5 plants had indices from 10 to 18 (av. 14.2), and 14 plants were *C. nutans* with a hybrid index of 19.

Index Area 18

Location: 4½ miles southwest of Flesherton, Conc. IN, lot 9, Artemesia Twp. About 50 plants were scattered, on top of a slight hill, in a pasture. Most of these plants were *C. nutans*, a few plants were hybrids tending towards *C. nutans* and no plants appeared to be typical *C. acanthoides*. Fifteen plants were indexed; 1 was hybrid with an index of 10 and the remaining 14 plants were *C. nutans* with an index of 19.

Index Area 19

Location: 3½ miles southwest of Flesherton, Conc. IIN, lot 19, Artemesia

Twp. Two ridges in this pasture were densely covered with *Carduus* plants which were less abundant on the surrounding low land. Almost all the plants were *acanthoides*-type hybrid segregates. Few *C. acanthoides* and no *C. nutans* were seen at this location. Twenty plants were indexed; 16 were hybrid with indices from 1 to 5 (av. 2.8), 1 hybrid had an index of 11, and 3 plants had the hybrid index of *C. acanthoides* (0).

Index Area 20

Location: 2 miles southwest of Flesherton, Conc. IIIN, lot 28, Artemesia Twp. Most of the plants in this low-lying pasture were *acanthoides*-type hybrids. A few plants about 100 yards from the main stand of *Carduus* were *nutans*-type hybrids. Twenty-four plants were indexed; 21 had indices from 1 to 6 (av. 3.9) and 3 plants had indices from 11 to 18 (av. 15.7).

Index Area 21

Location: 1 mile southwest of Flesherton, Conc. IIW, lot 151, Artemesia Twp. A large stand of *Carduus* occurred in a very hilly pasture about 300 yards long. Plants were most abundant on the higher areas and were generally thinly scattered elsewhere. Most of the plants seen were *acanthoides*-type hybrids with relatively few *C. acanthoides* and no *C. nutans*. Of 35 plants indexed, 30 were hybrids with indices from 1 to 6 (av. 2.7) and 5 plants had an index of 0.

Index Area 22

Location: 0.4 mile northeast of Flesherton, Conc. II, lot 150, Artemesia Twp. A large stand of *Carduus* in a gravel pit consisted chiefly of plants with low indices and a few typical *acanthoides*. No plants of *C. nutans* were seen at this location. Twenty-five plants were indexed; 19 were hybrids, indices 1–9 (av. 3.6), and 6 had the index of *C. acanthoides* (0).

Index Area 23

Location: ½ mile northeast of Flesherton, Conc. VII, lot 24, Artemesia Twp. A very large stand of *Carduus* was present in this pasture; these were mostly hybrids that had segregated toward *C. acanthoides*. Both parents were also present in this pasture. Of 25 plants indexed, 15 were hybrids with indices from 1 to 9 (av. 4.5), 3 plants were hybrids with indices from 11 to 17 (av. 14.0), 3 plants had the hybrid index of *C. acanthoides* (0), and 4 plants had an index of 19.

Index Area 24

Location: ¾ mile east of Rockmills, Conc. VI, lot 23, Artemesia Twp. All the plants examined in this old rocky pasture were typical *C. nutans*, which has probably been long established here.

Index Area 25

Location: 3.2 miles west of Maxwell, just before the bridge, Conc. VII, lot 37, Artemesia Twp. A large stand of *Carduus* in a gravel pit consisted chiefly of hybrids with a low index score. Both parents were also present at this location. Thirty-five plants were indexed; 22 were hybrids, indices 1–9 (av. 4.3), 4 hybrids had indices from 10 to 18 (av. 14.0), 3 plants had the index of *C. acanthoides* (0), and 3 plants had an index of 19.

Index Area 26

Location: 2 miles west of Maxwell, Conc. VI, lot 2, Osprey Twp. About 95% of the *Carduus* in this pasture was *C. nutans*; the remainder was made up about equally of *C. acanthoides* and hybrids approaching this species. A large well-established patch of *C. nutans* occurred in one part of the pasture with a small patch of *C. acanthoides* a short distance away. A few *acanthoides*-type hybrids were found growing between these two patches. Of 15 plants indexed, 2 were hybrids, indices of 2 and 3, 2 plants had the hybrid index of *C. acanthoides* (0), and 11 plants had the index of *C. nutans* (19).

Index Area 27

Location: 2½ miles east of Maxwell, Conc. VII, lot 23, Osprey Twp. All plants in this pasture were typical *C. nutans*.

Index Area 28

Location: 1.8 miles southeast of Holland Centre, Conc. IE, lot 41, Holland Twp. All plants in this stony pasture were typical *C. nutans*.

Index Area 29

Location: 7½ miles southeast of Holland Centre, Conc. IW, lot 49, Holland Twp. *Carduus* was very common in this pasture and all plants were *C. nutans*.

Index Area 30

Location: 1 mile southeast of Markdale, Conc. IE, lot 118, Artemesia Twp. There was a large stand of *Carduus nutans* in this pasture. A small patch of *C. acanthoides* and *acanthoides*-type segregates occurred towards the back of the pasture and between this patch and the road were many hybrids ranging from near-*acanthoides* to near-*nutans*. In the two pasture fields beyond this hybrid field were very large stands of *C. nutans*. Thirty plants in the pasture with hybrids were indexed; 9 were hybrids with indices from 2 to 8 (av. 3.7), 11 hybrids had indices from 13 to 18 (av. 15.3), 1 plant had the hybrid index of *C. acanthoides*, and 9 plants had the hybrid index of *C. nutans*. The proportion of *C. nutans* plants in this pasture was much larger than is indicated by the proportion among those indexed.

Index Area 31

Location: 2 miles southeast of Markdale, Conc. IW, lot 116, Artemesia Twp. A small patch of *C. nutans* occurred in a low area of this rolling pasture near the road. Next from the road were plants of *C. acanthoides* and *acanthoides*-type hybrids, then hybrids tending towards *C. nutans*, and, at the back of the pasture, were typical *C. nutans*. In the pasture beyond, farther from the road, and in the adjacent field to the west were large stands of typical *C. nutans*. Of 31 plants in the hybrid field indexed, 12 plants had indices from 1 to 9 (av. 5.2), 12 plants had indices from 10 to 17 (av. 12.2), 1 plant had an index of 0, and 16 plants had an index of 19.

Index Area 32

Location: 3 miles northwest of Flesherton, Conc. IE, lot 125, Artemesia Twp. A small number of plants occurred in a triangular patch at the back of a flat pasture. Most of these plants were hybrids; a few plants were *C. acan-*

thoides and *C. nutans*. Seventeen plants were indexed; eight were hybrids with indices from 3 to 9 (av. 5.6), seven were hybrids with indices from 11 to 18 (av. 14.0), one plant had the index of *C. acanthoides*, and one plant had the index of *C. nutans*.

Index Area 33

Location: 2 miles northwest of Flesherton, Conc. IE, lot 135, Artemesia Twp. *Carduus* plants were growing in a pasture with strong competition. Most of the *Carduus* plants were *C. nutans*, some were hybrids tending towards *C. nutans*, but none were typical *C. acanthoides*. Fifteen plants were indexed; one plant had an index of 9, seven plants had indices from 12 to 17 (av. 14.9), and seven plants had an index of 19.

Index Area 34

Location: ½ mile northwest of Flesherton, Conc. IW, lot 144, Artemesia Twp. A small stand of *Carduus* was growing in a sand–gravel pit. Most of the plants were *acanthoides*-type hybrid segregates; a few plants were typical *C. acanthoides*. Of 16 plants indexed, 15 had indices from 1 to 6 (av. 2.9), and 1 plant had an index of 0.

Index Area 35

Location: 1½ miles southeast of Flesherton, Conc. IW, lot 159, Artemesia Twp. This rocky pasture rises to a low ridge at the end away from the road. All of the plants had low indices. Of 18 plants indexed, 16 had indices from 1 to 5 (av. 2.7), and 2 plants had an index of 0.

Index Area 36

Location: 2¼ miles southeast of Flesherton, Conc. IW, lot 170, Artemesia Twp. Plants in this pasture were mostly hybrids phenotypically close to *C. acanthoides*. A small amount of *C. acanthoides* but no *C. nutans* was present in this field. Eighteen plants were indexed; 17 had indices from 1 to 7 (av. 3.8), and 1 plant had the hybrid index of *C. acanthoides* (0).

Discussion

It remains to attempt a synthesis of the foregoing data: to consider what areas were populated by each of the species prior to the initiation of hybridization; to determine at what points hybridization first occurred; whether hybridization is recurring commonly or whether only hybrids are now spreading, and whether there is any indication of the relative biological success of parental species and hybrids.

The observations recorded in Fig. 1 and specimens in the herbarium of the Department of Agriculture, Ottawa, show that pure stands of typical *C. nutans* are to be found in the southern portion of Grey County and in the counties immediately to the south. In Grey County, pure stands of *C. acanthoides* occur mainly north of Chatsworth. A few small stands were noted at a point about 9 miles south of Chatsworth. The species is very abundant in the area north and northwest of Owen Sound.

The area now occupied by hybrids lies within that portion of Grey County roughly south of a line drawn halfway between Chatsworth and Flesherton,

an area where pure stands of *C. nutans* are common but where no pure stands of *C. acanthoides* are known to occur. It is therefore concluded that *C. nutans* was first established in this hybrid area and that *C. acanthoides* entered at a later date and hybridization began. Examples of the manner in which this occurred can be seen in index areas 30 and 31. These pastures contained hybrids along the roadside but farther in from the road were occupied by pure stands of *C. nutans*, obviously long established. It was apparent that *C. acanthoides* had invaded these pastures from the road.

The white-flowered form of *C. acanthoides* (f. *albiflorus* (L.) Gross) is

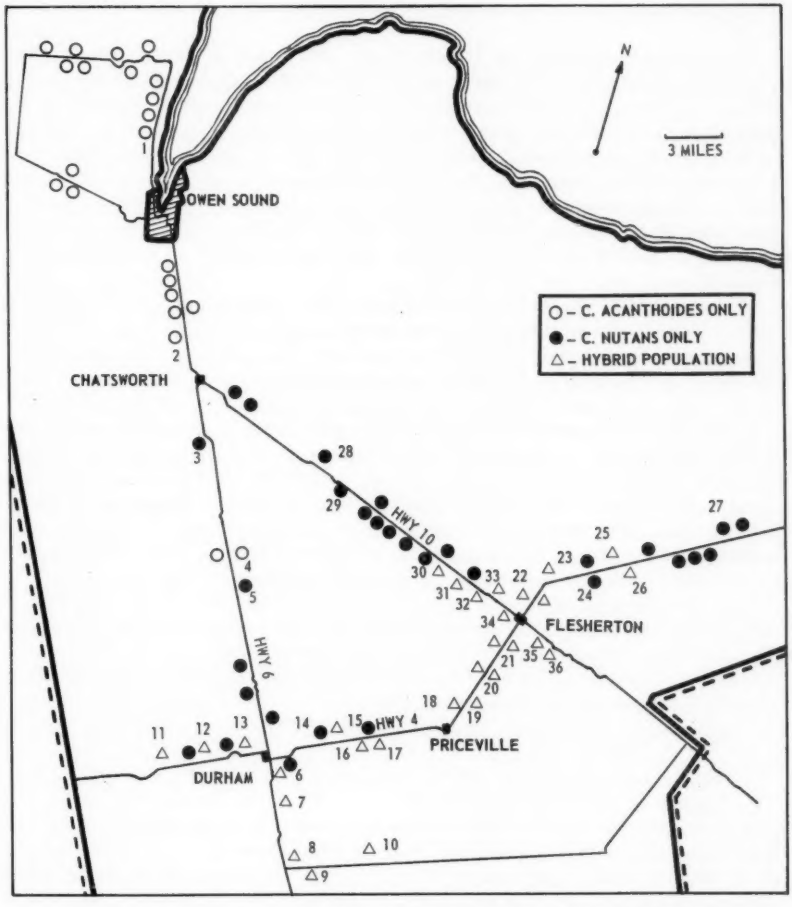

FIG. 1. Nature of *Carduus* populations in Grey County, including 36 fields indexed.

known to occur in Canada only northwest of Owen Sound. The white-flowered form of *C. nutans* has not been reported in Canada. White-flowered plants of *C. acanthoides* and of hybrids closely approaching this species were observed at index areas 19, 20, 21, 22, 30, and 36, all of which lie within a 5-mi radius of Flesherton. It seems, therefore, highly probable that the Flesherton area was invaded by a stock of *C. acanthoides* containing both white and purple-flowered plants from the infestation near Owen Sound. The absence of the white-flower character in fields west of Priceville indicates that the stock of *C. acanthoides* in this area was the result of a separate introduction of purely purple-flowered plants, probably also from the vicinity of Owen Sound. The spread of the white-flower gene will be a matter of interest for future study.

The great abundance of hybrid fields surrounding Flesherton suggests that this area is the focal point of hybridization and it is believed that *C. acanthoides* was first introduced here. Spread of the invader and hybrids has probably been facilitated by the use of gravel from pits for local road improvement and the gravel pit at index area 22 may have been an early source for distribution of seed. The abundance of hybrids of low index in the Flesherton area suggests that segregation has here been in progress for a considerable time. Probably *C. acanthoides* was then introduced into other gravel pits at areas 25 and 34 and from the pit at area 10 has spread in the area west of Priceville.

Both parental species and hybrids of intermediate type were found at areas 6, 9, 15, 26, 30, 31, and 32 and there seems little doubt that these are sites of recent primary hybridization. At area 11 a large recent invasion of *acanthoides*-type hybrid segregates has come into contact with a small stand of *C. nutans* and hybridization is beginning again in a yet restricted area.

At Index Areas 17, 18, and 33 were large stands of *C. nutans* with a relatively few hybrid plants, most of which approached *nutans* in appearance. These three fields, the only ones seen where most of the hybrids were morphologically close to *C. nutans*, were probably sites where extensive stands of *C. nutans* had been invaded by a small amount of *C. acanthoides* or by hybrids. The resulting or later generation hybrids segregated toward the species *nutans* by repeated backcrossing to this numerically superior species.

The development of the *Carduus* populations in the remaining areas is more difficult to interpret and in some cases more than one explanation is possible. The nature of these index areas may be summarized as follows:

Large populations of *acanthoides*-type hybrids and relatively few plants of both parental species—23, 25.

Populations of hybrids with *C. acanthoides* only—7, 10, 19, 20, 21, 22, 34, 35, 36.

Populations of hybrids with *C. nutans* only—12, 13, 16.

Populations of hybrids only—8.

The surveys of the Priceville field have shown that a population containing both parental species and hybrids may, in the course of 5 years, be replaced by a wholly hybrid population, the majority of plants of which approach the species *acanthoides* in morphology. A similar transformation has probably

taken place in some of the above fields, resulting in the disappearance of one or both parental species. There is also the possibility, however, that, in some cases, hybrids did not arise in the fields where they are now found. Pastures occupied by hybrids and one parental species may earlier have been occupied by the species and has since been invaded either by hybrids or by small stock of the other species which failed to persist in the face of numerical superiority. Since the hybrid zone lies in *nutans* territory—where old, pure stands of *acanthoides* are unknown—this generalization will apply only to fields of *nutans* and hybrids. It is concluded that fields containing a large stand of *C. nutans* and a relatively few hybrids of low index score (areas 12, 13, 16) are fields of *nutans* which have recently been invaded by *acanthoides*-type hybrids.

Dense populations of hybrids closely approaching *C. acanthoides* in appearance were repeatedly observed. These plants differed from typical *acanthoides* by one or more of the following characters: peduncles not spiny winged to the head; phyllaries slightly reflexed; purplish marking on the tip of the outer phyllaries absent. These hybrid segregates scored in the range 1 to 5. The white-flower character, when present, was always associated with these phenotypes. Such plants constituted almost the entire population of fields 7, 8, 9, 11, 19, 20, 21, 22, 34, 35, and 36. In all except area 8, a few plants of apparently pure *acanthoides* occurred among the hybrids of low score. Whether these typical plants were remnants of the invaders that initiated hybridization, or were ultimate products of segregation toward the *acanthoides* phenotype cannot be decided. If the latter is the case, it is possible that such apparently typical *acanthoides* plants may still carry some *nutans* genes, if not as whole chromosomes then as segments derived through crossing over, and may be physiologically different from the original *acanthoides*.

From the numerical supremacy of such hybrids of low index score, both in the number of individuals per field and in the frequency of fields occupied by such plants, it is concluded that segregation toward the *acanthoides* genotype is favored by selective factors. There is every indication that such hybrids are better adapted to southern Grey County than is *C. nutans* and will supplant this species. It is probable that the chief spread of the hybrid will be effected by these *acanthoides*-type segregates.

References

1. GILLESPIE, J. E. and RICHARDS, N. R. 1954. Soil survey of Grey County. Rept. 17 Ontario Soil Survey, Guelph, Ont. 1–79.
2. MULLIGAN, G. A. and FRANKTON, C. 1954. The plumeless thistles (*Carduus* spp.) in Canada. Can. Field-Naturalist, **68**, 31–36.
3. MOORE, R. J. and MULLIGAN, G. A. 1956. Natural hybridization between *Carduus acanthoides* and *Carduus nutans* in Ontario. Can. J. Botany, **34**, 71–85.

DORMANCY STUDIES IN SEED OF AVENA FATUA

2. A GIBBERELLIN-SENSITIVE INHIBITORY MECHANISM IN THE EMBRYO[1]

J. M. NAYLOR AND G. M. SIMPSON

Abstract

Natural inhibition of germination in *A. fatua* L. involves restriction of both sugar accumulation and utilization of sugar in growth. Both these metabolic blocks are independently overcome by exogenous gibberellic acid (GA). Evidence has been found for the presence of a natural gibberellin in seeds of *A. fatua* L. The control of dormancy is probably by a gibberellin-inhibitor antagonism since at least one inhibitor has been shown to intervene specifically in sugar production and this latter effect can be reversed by GA. The evidence presented here supports the view that control of germination during the period of after-ripening is through changes in inhibitor content rather than in endogenous gibberellin.

Introduction

The experiments reported here concern the factors influencing germination of dormant embryos of wild oats (*Avena fatua* L.). Previous investigations have shown that seed dormancy in this species, as in others such as *Xanthium*, can be broken by holding imbibed seeds in an oxygen-rich atmosphere (9). Evidence that, as in *Xanthium* (10), oxygen is necessary for the destruction of a germination inhibitor has been presented by Black (2). More recently (3) it has been found that the onset of dormancy during seed maturation can be prevented by allowing maturing inflorescences to take up gibberellic acid (GA). These findings suggest that the prevention of dormancy by this compound reflects antagonism between an endogenous GA-like substance and an inhibitor. Such an interpretation of the action of GA in other growth phenomena has been suggested previously (4). Supporting evidence has been provided by Simpson (8).

The following experiments were conducted to provide information on the nature of the GA effect in germination, and in particular to investigate the possibility that germinating capacity is controlled by a gibberellin-inhibitor system.

Methods

Embryos from a uniform inbred line of *A. fatua* L. were used in these experiments. The following procedure was used in preparing material for germination tests. Seeds were allowed to imbibe water for about 15 minutes. The embryos, with a thin layer of adhering endosperm, were excised with a sharp scalpel under a dissecting microscope. Air-dry embryos were transferred in lots of 50 to pieces of Whatman No. 1 chromatography paper ($1\frac{1}{2} \times \frac{7}{8}$ in.) in small Petri dishes. Papers were wetted with 0.33 ml of the treatment solutions. Each treatment consisted of two such replicates, except in

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some experiments where three replicates of 33 embryos per treatment were used. In the latter, paper size and volume of solutions were adjusted proportionately. In experiments where the period of incubation was expected to extend over 5 or more days, the medium was supplemented with the antibiotics candidin and neomycin, in concentrations of 250 and 20 p.p.m. respectively. In all experiments, embryos were incubated in a humid atmosphere at 20° C in darkness.

Seed extracts used in assays for endogenous gibberellins were prepared as follows. Two replications, each of 1300 seeds, were macerated for 30 minutes in 200 ml of 70% ethanol at 1–4° C. After filtration, the solid residue was stirred with a further 200 ml aliquot of ethanol for 2 hours at 1–4° C. After combining the filtrates, ethanol was removed under reduced pressure (max. temperature, 32° C) and the water residue acidified to pH 3.0 with *N* HCl. The acidified extract was shaken three times with 130-ml aliquots of ethyl acetate. The ethyl acetate was in turn shaken with four 100-ml aliquots of KH_2PO_4–KOH buffer (pH 6.2). Following acidification to pH 3.2 with HCl, the buffer was extracted three times with 100-ml aliquots of ethyl acetate. Water was removed from the final ethyl acetate fraction by chilling to −15° C and filtering. This final fraction, which contained acidic substances, was reduced to small volume (1 ml) for chromatography.

Determination of sucrose in plant tissue was as follows. Water extracts containing sugars were prepared by refluxing 70% ethanol over the tissue for 1 hour at 80° C, followed by removal of the alcohol under reduced pressure. Quantitative estimations of sucrose were then made by the method of Edelman *et al.* (5). Quantitative estimations of the dye obtained with the resorcinol reagent were made with a Bausch and Lomb colorimeter against standard solutions.

Gibberellic acid, melting point 232–233.5° C, was supplied by Imperial Chemical Industries Ltd.

Results

Preliminary experiments indicated that newly matured caryopses were not induced to germinate by breaking the seed coats prior to imbibition in air. However, when the seed coats were broken, germination did occur in pure oxygen. This result indicated that the control of dormancy was not dependent entirely on the restriction of gas exchange by the seed coats, as previously supposed (1), but that these embryos may have a peculiarly high requirement for oxygen during germination.

Evidence that seed dormancy is, in fact, a function of the physiological state of the embryo was found in the following experiment. Embryos excised from seeds which had been held in dry storage for various periods up to 3 years were germinated on moist filter paper. As shown in Fig. 1, both the rate of germination as well as the final proportion of germinable embryos increased with period of storage. This indicated clearly that true embryo dormancy exists in the species, and that embryos gradually recover the capacity to germinate in air, over a period of at least 2 years. This increase in capacity for germination during storage suggested that some essential metabolic process is suppressed in the dormant embryo. Moreover, the onset

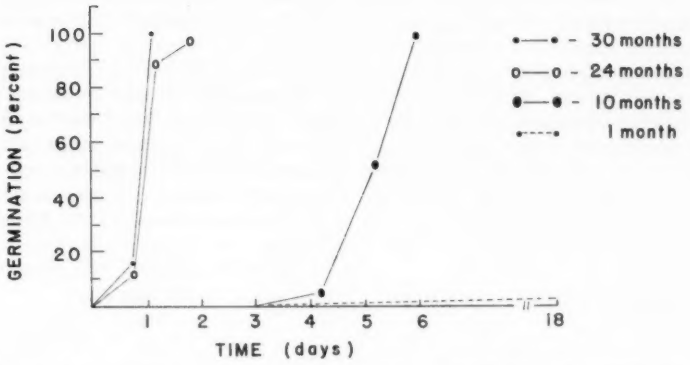

FIG. 1. Influence of age, after maturity, on rate of germination of excised embryos.

of this inhibition can be prevented by the administration of GA (3) during seed maturation. The basis of this GA effect is not clear, though recent findings by Paleg (7) have shown that GA induces the production of sugar from endosperm starch in barley seeds. Accordingly, it was of interest to determine whether the availability of sugar is the limiting factor in germination of dormant embryos, and whether the stimulation of germination by GA could be attributed primarily to the release of sugar from endosperm starch.

The results presented in Figs. 2 and 3 indicate the change in response to GA during after-ripening. Embryos used in these experiments were taken from seeds of the inbred line stored for 1 month (Fig. 2), and 24 months (Fig. 3). In the former, rate of germination induced increased with increase of concentration of GA over the range (0.02–50) p.p.m. In the latter, germination occurred much more promptly and in response to a lower range of concentration (0.001–0.1) p.p.m. These results indicate that the administration of GA at concentrations of 0.1 p.p.m. or higher is sufficient to induce germination of even newly matured embryos. Moreover, although "old" embryos germinated without GA, the rate was increased by relatively small amounts of this substance.

Response to exogenous sucrose was investigated in a set of experiments similar to those described above. Embryos were excised from seeds stored for periods of 5 and 18 months and supplied with sucrose in concentrations of 2, 5, and 10% (Fig. 4). The results are particularly interesting, since exogenous sucrose had strong promotive effect on germination in the seeds stored for a short period, but slightly delayed germination in those stored for 24 months. This suggested that in newly matured dormant embryos insufficient endogenous sugar was available for germination and that this limitation is overcome during after-ripening. The lack of response to exogenous sucrose on the part of "old" embryos might reflect an accumulation of sugar in endosperm or embryo during after-ripening, or a greater capacity to form sugar after imbibition. In any case, the requirement of dormant

embryos for exogenous sucrose in germination can be replaced by GA. This provides the corollary to Paleg's finding (7) that GA induces the conversion of starch to sugars in endosperm.

The preceding experiments indicate also that the effect of GA in promoting germination cannot be attributed entirely to an effect on the production or translocation of sugars. Comparison of Figs. 3 and 4 show that "old" embryos which germinated when supplied only with water, nevertheless responded to small doses of GA, but not to sucrose. The extent to which sucrose can substitute for GA in relieving dormancy was tested in the same experiment in treatments involving sucrose alone, and in combination with GA. Although

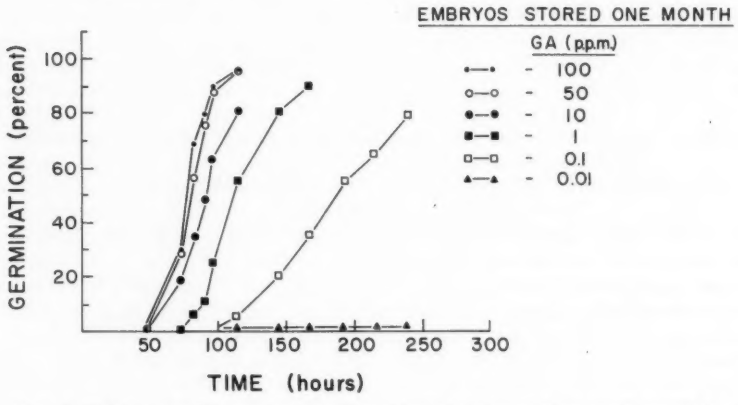

FIG. 2. Influence of GA on rate of germination of embryos from seeds stored for 1 month after maturity.

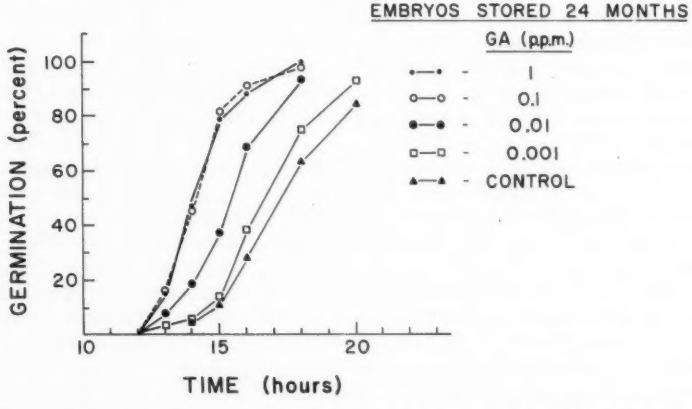

FIG. 3. Influence of GA on rate of germination of embryos from seeds stored for 2 years after maturity.

the optimal concentration of sucrose was not determined, it can be assumed to be less than 10% (Fig. 4), since the onset of germination with the 5% treatment was somewhat earlier. (Times required for 50% germination: 5% sucrose 58.0 hours; 10% sucrose 63.5 hours). Nevertheless, there was a marked response to GA in combination with 10% sucrose (Fig. 5). Since this effect could not have resulted from the formation of additional sucrose, GA must have a second promotive effect not related to sugar production. In comparing the germination curves for these treatments it was noted that the effects of GA and sucrose on rate of germination were nearly additive (Table I). Mean germination times for the treatments were calculated when 100% of the treatment embryos had germinated and the untreated control embryos

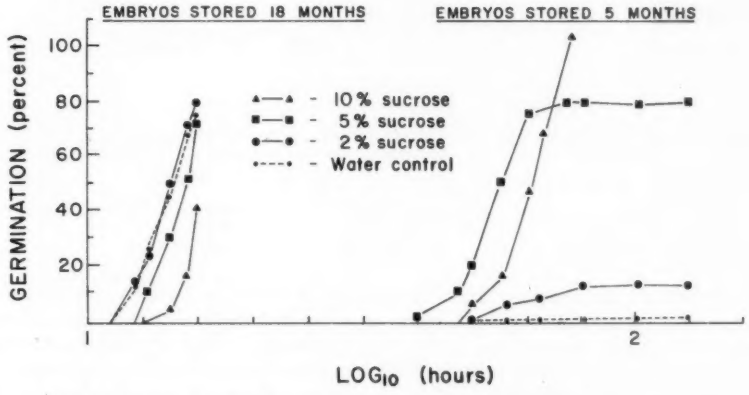

FIG. 4. Influence of age after maturity on response to exogenous sucrose.

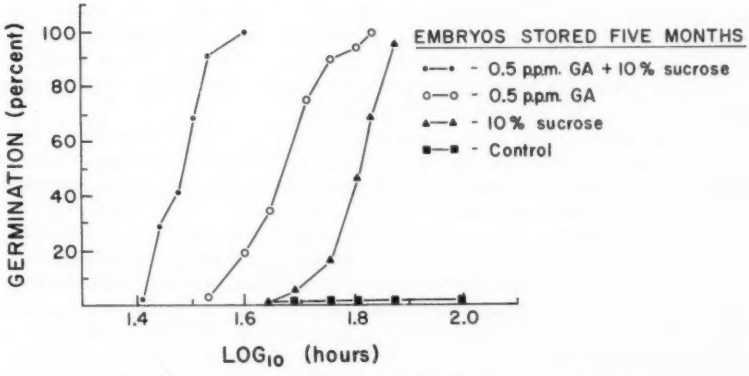

FIG. 5. Influence of exogenous sucrose and GA on rate of germination of embryos from seeds stored for 5 months after maturity.

TABLE I

Additive effect of GA and endogenous sucrose (S) in decreasing the mean germination time of excised embryos of *A. fatua* L.

Treatment	T_{GA}	T_S	T_{SGA}	$1/T_{GA} + 1/T_S$	$1/T_{SGA}$
GA (0.5 p.p.m.)	49.09 *(0.36)				
Sucrose (10%)		63.55 (1.49)			
Combination			30.67 (0.69)	0.0361	0.0326
GA (1 p.p.m.)	44.09 (1.38)				
Sucrose (10%)		63.55 (1.49)			
Combination			29.90 (1.51)	0.0384	0.0334
GA (10 p.p.m.)	39.44 (2.46)				
Sucrose (10%)		63.55 (1.49)			
Combination			28.15 (0.25)	0.0411	0.0355

T = Mean germination time in hours, per embryo.
$1/T$ = rate of germination.
* = Standard error of mean.

remained ungerminated. Since the embryos used in this experiment had matured 5 months prior to the experiment and therefore might have undergone some changes in state of dormancy during storage, it was not known whether the observed relationship represented only a transitory stage in after-ripening, or whether GA and sucrose exhibit additive effects also in embryos at the time of maturity. Accordingly the influence of age of seed on interaction of sucrose and GA was examined further. It was of particular interest to determine whether there is GA-sucrose synergism in germination of newly ripened embryos. Thus it was desirable to administer the maximum concentration of GA, which, in the absence of exogenous sucrose, would have little or no stimulating effect on germination. A preliminary experiment indicated this concentration to be approximately 0.1 p.p.m.

Embryos were excised from seeds which had been stored following maturation for 1 week, 7 months, and 24 months. These were incubated on papers wetted with (*a*) 5% sucrose, (*b*) 0.1 p.p.m. GA, (*c*) a combination of sucrose and GA in the above concentrations. Solutions were made up in the usual mixture of antibiotics. All treatments involved three replicates of 33 embryos each. Embryos in each replicate were placed on three layers of paper (1 × $\frac{7}{8}$ in.), and supplied with 0.48 ml of solution. The results plotted in Fig. 6, representing newly matured embryos, indicated only a slight response to either GA or sucrose, though germination in the combination treatment was complete in 96 hours.

Embryos which had matured 7 months prior to the experiment responded to either GA or sucrose (Fig. 7). However, the effects were less than additive

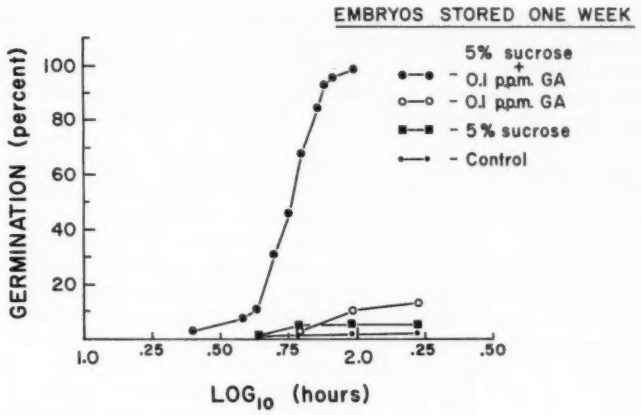

FIG. 6. Influence of exogenous sucrose and GA on rate of germination of embryos from seeds stored for 1 week after maturity.

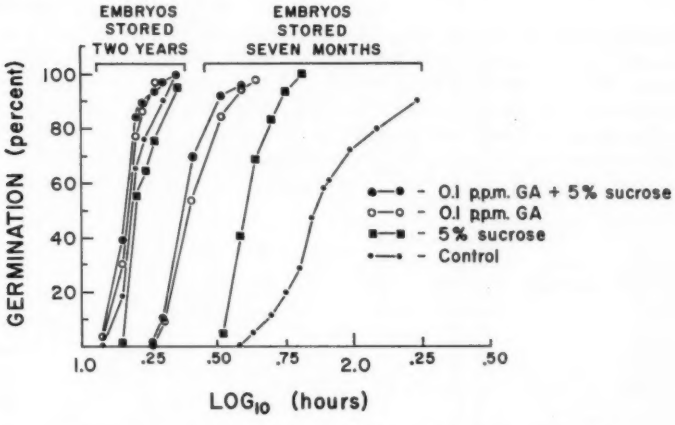

FIG. 7. Influence of age after maturity on response of excised embryos to sucrose and GA.

since GA alone was nearly as effective as the combination treatment. In 2-year-old embryos all treatments had relatively slight effect on germination rate.

Failure of the 5% sucrose or 0.1 p.p.m. GA treatments to stimulate germination of embryos previously stored 1 week suggested that either the supply of sucrose was exhausted or that the embryos were unable to utilize sugar for growth. To clarify this the following experiment was carried out.

TABLE II

Utilization of sucrose by newly ripened excised embryos during 96-hour germination period

Treatment	Germination (%)	Sucrose (mg) per replicate present in:					
		embryos		papers		total	
		Initially	Finally	Initially	Finally	Initially	Finally
Sucrose	7.07	2.45† (0.10)	8.12 (0.87)	23.94 (0.87)	7.00 (0.43)	26.39	15.12
Gibberellic acid	10.10	2.45 (0.10)	—	—	—	2.45 (0.10)	—
Sucrose + gibberellic acid	100.0	2.45 (0.10)	9.24 (0.80)	23.94 (0.87)	12.25 (0.63)	26.39	21.49

Values in parentheses = standard error.
†Value for 2-year-old embryos = 2.10 (0.15).

Newly matured embryos were incubated on 5% sucrose alone, and in combination with 0.1 p.p.m. of GA as described above. After 96 hours when germination in the latter treatment was complete, embryos were removed, and the residual sucrose in the papers and embryos was measured using the method of Edelman *et al.* (5). The initial sucrose content of the embryos was also determined.

It was found (Table II) that, when sucrose alone was supplied, more than half of the initial endogenous plus exogenous sucrose supply was present in the embryos and paper after 96 hours. Moreover, the amount of sucrose finally contained in the embryos was not significantly less than in those embryos which underwent germination in the combination treatment. Evidently failure of germination in the former case was not caused by an inadequate supply of sucrose, but, rather, by the inability of dormant embryos either to metabolize this sugar at a sufficient rate to support germination, or to utilize energy from this source specifically in growth. The fact that this limitation can be overcome by small amounts of GA (Fig. 6) accounts for the observed sucrose–GA synergism.

It is interesting that the net depletion of sucrose in the combination treatment was slightly less than 20% whereas in the sucrose treatment it was almost 50%. This may reflect a sugar-sparing effect of GA, or a GA-enhanced rate of conversion of starch to sucrose.

Another feature of the interaction of GA and sucrose is demonstrated in Fig. 8. With newly ripened embryos the dose–response curve for GA alone shows a maximum at approximately 100 p.p.m. Where 5% sucrose was supplied, germination occurred in response to a lower range of concentrations, with a maximum of 0.1 p.p.m. It is of interest that the maximum effective dose of GA in 18-month-old embryos was also close to the above, with or without exogenous sucrose. This establishes the participation of GA in at

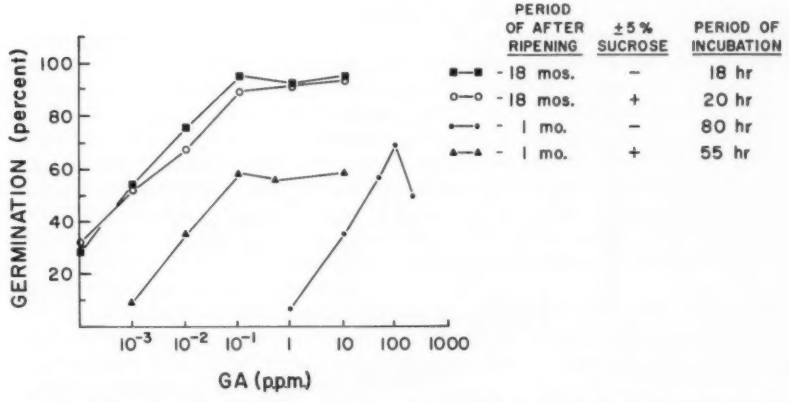

FIG. 8. Influence of exogenous sucrose and age after maturity on dose response to GA. Largest standard error of points shown = ± 2.2.

least two processes in germination, those of sugar production and sugar utilization in growth. Since the maxima differ by a factor of about 1000 in GA concentration, it is apparent that, of the two, the process involving sugar production has a relatively much greater requirement for GA. It is not known whether rate of germination of *A. fatua* embryos becomes entirely independent of exogenous GA during a longer period of after-ripening. In any case, embryos of the domestic variety Torch (*A. nuda* L.) showed little or no response to GA after 3 years, though recently matured embryos exhibited a strong dose response (Fig. 9).

Evidence that endogenous germination inhibitors are important factors in seed dormancy in this species has been presented elsewhere by Black (2). Because a natural gibberellin occurs in many seeds, including those of *A. fatua* (see below), it was considered that such a substance might promote germination by reversing the effects of an inhibitory substance. Since these inhibitors have not been identified or isolated in quantity, this possibility could not be tested directly. However, if leaching dormant embryos in water improved their capacity to germinate, this would constitute evidence of the removal of an inhibitory water soluble substance. Moreover, enhancement of response to GA by such treatment would suggest antagonism between the inhibitor and GA.

The effects of leaching were tested as follows. Two hundred newly ripened embryos were placed on filter paper in a Buchner funnel. Distilled water was allowed to drip through the paper at the rate of 6 ml per hour. An equal number of embryos were held fully imbibed (in lots of 100) on two small papers ($1\frac{1}{2} \times \frac{7}{8}$ in.), each moistened with 0.4 ml of water. These conditions were maintained for 5 days at 20° C in darkness. Embryos of each treatment were then transferred, in groups of 50, to standard germination papers, wetted with 0.23 ml of solution containing 5% sucrose alone or in combination with 0.005 p.p.m. of GA.

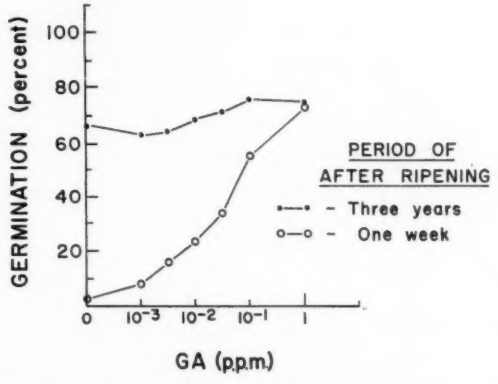

FIG. 9. Influence of age after maturity on response of Torch embryos to exogenous GA.

The results (Table III) indicated that the response to both GA or sucrose was increased by leaching. This experiment suggested that the removal of an inhibitory substance from dormant embryos by this treatment had an effect similar to that of after-ripening.

It was found in a previous experiment that one of the changes occurring during normal after-ripening was an increase in capacity of a given dose of GA to satisfy the sugar requirement of the embryos in germination. For example, 0.1 p.p.m. of GA did not induce germination of newly ripened embryos, though it was effective in the presence of adequate sucrose (Fig. 6). On the other hand, this dose of GA was nearly as effective as the combination treatment in partially after-ripened embryos (Fig. 7). The following experiment was set up to test whether a similar increase in capacity of GA to substitute for exogenous sucrose could be induced by leaching.

TABLE III

Effect of leaching on germination of dormant excised embryos

Pretreatment	Replicate*	Germination (%) after 44 hours	
		5% sucrose	5% sucrose, 0.005 p.p.m. GA
Leached in large volume	1	50	92
	2	52	86
Imbibed	1	19	23
	2	15	18

*Each replicate consisted of 50 embryos.

Four hundred newly ripened embryos were placed on a circular piece of filter paper of 15-cm diameter, supported by a glass disk of the same size. Distilled water was allowed to drip onto the paper at the rate of 25 ml/hour, for 48 hours. An equal number of embryos were held fully imbibed but with only slight excess of water, for the same period. On transfer the following treatments were given—0.1 p.p.m. GA, 0.1 p.p.m. GA with 5% sucrose, control (with neither compound). All solutions contained the antibiotics in the usual concentrations. Each treatment consisted of three replicates of 33 embryos on two thicknesses of paper, each 1 × $\frac{1}{4}$ in., wetted with 0.35 ml of solution. The results are presented in Fig. 10. No germination occurred in the control treatment of either group during 96 hours. Leaching did not increase greatly the rate of germination in the combination treatment; however, it increased substantially the rate of germination in the presence of GA alone. That is, the capacity of a given quantity of GA to substitute for sucrose was increased by the leaching treatment. As indicated above, this effect is similar to that of normal after-ripening.

The sensitivity of dormant embryos to exogenous GA, and the evidence that this compound influences germination by antagonizing the effects of endogenous inhibitory substances suggests that a natural gibberellin may have a promotive role in germination.

The presence of germination-promoting substances on two-dimensional paper chromatograms of tissue extracts should be revealed by the germination

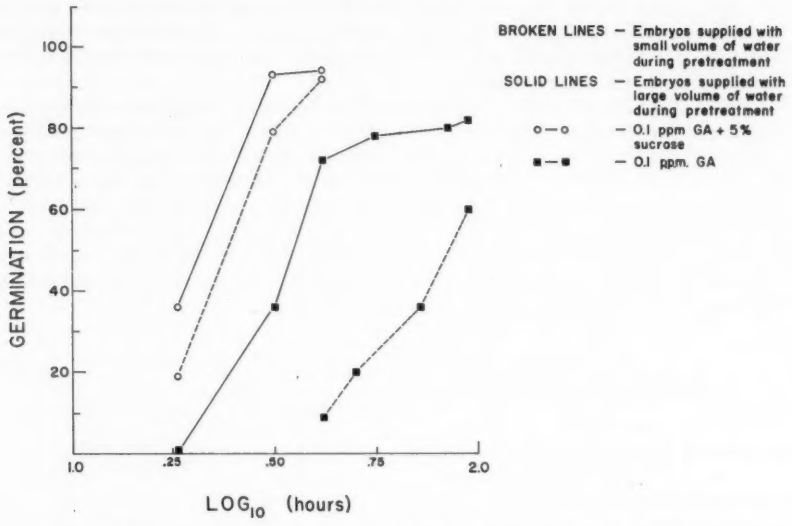

FIG. 10. Effect of leaching on response of excised embryos to GA and sucrose.

response of dormant embryos. If natural gibberellins occur in wild oats, positive responses to tissue extracts of caryopses would occur at R_f's corresponding to those of authentic GA. This was confirmed in the following experiment.

Extracts of two lots of 1300 2-year-old caryopses were prepared as outlined previously. Each extract was transferred to one corner of a square Whatman No. 1 chromatography paper and developed for 25 cm in solvent system A (isopropanol/ammonia (0.880)/water 10:1:1) where GA was located at R_f 0.62. After drying, the chromatogram was further developed for 18.5 cm in solvent B (isoamyl alcohol / ammonia (0.880) / water 110:30:62) at right angles to the first solvent front. Four adjoining segments of the chromatograms, each $\frac{7}{8} \times 1\frac{1}{2}$ in., were taken for bioassay, viz.:

(1) the correct position corresponding to authentic GA for both developments;
(2) correct position for GA in second development, but at a lower R_f value for the first development (1 and 2 were expected to contain all the GA in the extract);
(3) correct position for GA in first development but a higher R_f value for the second;
(4) lower R_f value in first development and higher than GA in second (3 and 4 constituted controls for the chromatogram and would not be expected to contain any GA).

Fifty *A. fatua* embryos were placed on each of the above papers. Each paper was wetted with 0.23 ml of 5% sucrose and antibiotic. Germination percent-

ages were recorded at 48 hours (Table IV). Segment No. 2 in each chromatogram shows a highly significant germination response of a similar magnitude to that induced by 10^{-2} p.p.m. in the control segments. A positive response in segment No. 2 is indicative of endogenous GA because exogenous GA chromatographed in the presence of natural extracts would be expected to show slightly lower R_f value in the first development due to the presence of impurities, and correct values in the second development. These results indicate that a substance with similar R_f's to GA in two solvent systems can be obtained from the caryopses of *A. fatua*.

TABLE IV

Assay for gibberellin-like substances in extracts of 2-year-old caryopses

	Germination (%) replications*	
	A	B
Chromatogram segment		
1	46	46
2	84	86
3	50	54
4	56	48
Non-chromatographed paper controls		
5% sucrose	40	42
0.01 p.p.m. GA + 5% sucrose	92	—

*Each replicate consisted of 50 embryos.

Discussion

Earlier interpretation of the control of seed dormancy in *A. fatua* assigned particular significance to the restriction of gas exchange by the seed coats (1, 6). This was justified chiefly by the fact that germination could be induced by rupturing the seed coats or by holding intact, imbibed seeds in an oxygen-enriched atmosphere. The essential change occurring in after-ripening, by which the seed gains the capacity to germinate in air, was thought to be an increase in seed coat permeability. The possibility that the oxygen-requirement of the embryo might decrease with time was not investigated, though subsequently this change was found to be the basis of after-ripening of *Xanthium* seeds. The present results show that physiological changes in the embryo account primarily for the loss of dormancy also in *A. fatua*.

From the data presented in this paper it is clear that newly matured embryos cannot mobilize sugar from endosperm reserves at a rate sufficient to support germination. This is undoubtedly an important factor in their dormancy. The response to exogenous sugar disappears during after-ripening (Fig. 4). This may reflect either the slow accumulation of sugars in the embryo and adjacent endosperm or an increased capacity to form sugar after imbibition. With regard to the former alternative, experimental evidence (Table II), indicates that there is no increase of endogenous sucrose in embryos during after-ripening, though the possibility that significant changes occur in the amount of other sugars cannot be excluded.

The fact that GA alone, in high concentrations, invariably induced germination indicates that it satisfied the sugar requirement of dormant embryos. This agrees entirely with Paleg's finding that GA increases the rate of conversion of starch to sugar in barley endosperm (7). Evidence that leaching dormant embryos increases the capacity of a given dose of GA to substitute for exogenous sucrose strongly suggests that GA reverses the effects of an endogenous substance which inhibits sugar production. This prevention of sugar production and, thus, the maintenance of starch reserves, provides an explanation of the well-known persistency of wild oat seeds in cultivated soils.

While the inhibition of sugar formation is obviously important, it is not the only factor contributing to embryo dormancy. This is evident since newly matured embryos could not be induced to germinate by sucrose alone. The basis of this inhibition of growth is not known, except that it, too, can be overcome by GA (Fig. 6). That GA has a second promotive effect not related to sugar formation is indicated by (1) GA–sucrose synergism (Fig. 6) and (2) the fact that rate of germination is GA dependent, even in "old" embryos which do not respond to exogenous sucrose (Fig. 4), and in newly matured embryos supplied with sucrose (Figs. 6 and 8).

These results do not show whether both of the observed effects of GA depend on GA-inhibitor antagonism, nor is there any indication of which metabolic processes other than sugar formation can respond to this substance. In any case, the two processes have distinctly different concentration optima for GA (Fig. 8). Moreover, of the two effects of GA on rate of germination, that involving sugar production disappears relatively earlier in after-ripening. This may reflect the existence of two GA-sensitive inhibitors which are lost or inactivated at different rates.

Evidence that loss of dormancy is correlated with decrease in activity of at least one inhibitory substance in intact caryopses was previously shown by Black (2).

In view of the evidence that an endogenous gibberellin occurs in *A. fatua* seeds, and since exogenous GA alone is capable of inducing germination or accelerating the rate of germination in embryos showing all degrees of dormancy, it appears likely that an endogenous gibberellin has a promotive role in this process. Thus it is necessary to consider whether loss of dormancy in after-ripening could be explained by an increase in amount, or activity, of an endogenous gibberellin. Such an increase might account for the lack of response of old Torch embryos to exogenous GA (Fig. 9). It would not, however, account for the loss of dormancy in *A. fatua* for the following reasons. Two-year-old embryos still respond to small amounts of GA (<0.1 p.p.m.) though not to sucrose (Figs. 3 and 4), which implies there is still a deficiency of GA. The requirement of newly ripened embryos for sugar was satisfied by GA only in concentrations greater than 0.1 p.p.m. (Fig. 8), whereas this concentration was nearly optimal for the other GA-sensitive process involving utilization of sugar in germination (Fig. 8). Thus any increase in concentration of endogenous GA sufficient to meet the demand for sugar production must be expected to satisfy the GA requirement for both processes.

Further evidence that changes in endogenous gibberellin are not significant in termination of dormancy is provided by the results of the leaching experiments. Leaching dormant embryos increased the rate of germination in the presence of a given small amount of GA. Response to sucrose was also increased by this treatment. If loss of dormancy was caused simply by an increase in endogenous GA, leaching would have the reverse effect, since some loss of GA could be expected.

It appears more likely that rate of germination is determined by the relative amounts of endogenous gibberellin and inhibitor. This is supported by the evidence that leaching increases the promotive effect of a unit quantity of GA (Fig. 10). Natural loss of dormancy may, therefore, depend principally on the decrease in the endogenous inhibitor (cf. Black (2)) which antagonizes GA. The finding that the germination inhibitor coumarin competitively reverses the promotive effect of GA in germination of *A. fatua* embryos (Naylor and Simpson, unpublished) may be relevant. An inhibitor–gibberellin antagonism in the control of growth is probably not confined to dormancy. This is indicated by the participation of a gibberellin–inhibitor mechanism of growth control in pea stem internodes (8).

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References

1. Atwood, W. M. 1914. A physiological study of the germination of *Avena fatua*. Botan. Gaz. **57**, 386–414.
2. Black, M. 1959. Dormancy studies in seed of *Avena fatua*. 1. The possible role of germination inhibitors. Can. J. Botany, **37**, 393–402.
3. Black, M. and Naylor, J. M. 1959. Prevention of the onset of seed dormancy by gibberellic acid. Nature, **184**, 468–469.
4. Brian, P. W. 1959. Effects of gibberellins on plant growth and development. Biol. Revs. **34**, 37–84.
5. Edelman, T., Shibko, S. I., and Keys, A. T. 1959. The role of the scutellum of cereal seedlings in the synthesis and transportation of sucrose. J. Exptl. Botany, **10**, 178–189.
6. Johnson, L. P. V. 1935. General preliminary studies on the physiology of delayed germination in *Avena fatua*. Can. J. Research, C, **13**, 283–300.
7. Paleg, L. G. 1960. Physiological effects of gibberellic acid: 1. On carbohydrate metabolism and amylase activity of barley endosperm. Plant Physiol. **35**, 293–299.
8. Simpson, G. M. 1959. Studies on the occurrence and distribution of certain growth hormones in plants. Ph.D. Thesis, London University.
9. Thornton, N. C. 1935. Factors influencing germination and development of dormancy in cocklebur seeds. Contribs. Boyce Thompson Inst. **7**, 477–496.
10. Wareing, P. F. and Foda, H. A. 1957. Growth inhibitors and dormancy in *Xanthium* seed. Physiol. Plantarum, **10**, 266–280.

GROUPING OF ISOLATES OF A LOW-TEMPERATURE BASIDIOMYCETE ON THE BASIS OF CULTURAL BEHAVIOR AND PATHOGENICITY[1]

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Abstract

Isolates of an unidentified low-temperature basidiomycete, associated with snow mold in Western Canada, were divided into three types, A, B, and C, on the basis of their general cultural appearance. Support for this classification was obtained when representative isolates of each type were examined to determine: the effect of temperature and pH on growth; tolerance of antibiotics and HCN; ability to liberate HCN in culture and in the host plant; pathogenicity.

Type A isolates grew slowly under most conditions and were least tolerant of the extremes of temperature and pH employed, moderately inhibited by antibiotics, and strongly inhibited by HCN. They produced HCN in large quantities in host plants and none in culture. They were moderately pathogenic on grass and highly pathogenic on alfalfa. Type B isolates grew somewhat more rapidly than type A, especially at the upper and lower temperatures, and grew over a wider pH range. These isolates were more tolerant of antibiotics and HCN. They produced smaller quantities of HCN than type A in infected alfalfa plants but released large amounts in culture. They were less pathogenic than type A on alfalfa but similarly pathogenic on grass. Type C isolates were fast-growing forms which were strongly inhibited by antibiotics and HCN. They did not liberate HCN under any conditions and were not pathogenic.

Introduction

A basidiomycetous fungus causing snow mold of turf grass in Alberta was first described by Broadfoot (1). The same fungus was later found to cause a severe winter crown rot of legumes (2) and its pathogenicity on a variety of species of grasses and legumes has been demonstrated under natural (4, 5, 6) and artificial (7) conditions. Fruiting bodies or spores have not been found either on diseased plants or in laboratory culture, but the ability to grow at low temperatures and the presence of hyphal clamp connections has led to the use of the term 'low-temperature basidiomycete' in describing the fungus (2). Lebeau and Dickson (11, 12) reported that HCN was released by cultures growing on laboratory media and obtained evidence that this substance was produced during infection of alfalfa plants, accumulating in crown tissue in concentrations highly toxic to the host plant. These findings have been confirmed by subsequent experiments (9, 10) and HCN production is believed to be the major factor in the etiology of diseases caused by this fungus.

Isolates have been collected from several hosts and from widely separated locations in Western Canada (5) and Alaska (13). These were identified as the low-temperature basidiomycete by their association with host material beneath snow cover in early spring and by their growth in culture at low

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temperatures, where they produced white sterile mycelium with typical clamp connections. However, examination of these isolates indicated that although they were generally similar they frequently differed in cultural appearance and other characteristics and could not be regarded as a completely homogenous group. In this paper a number of observations and experiments are described which illustrate these differences and provide a basis for a simple classification of the isolates.

Results

Cultural Appearance of Isolates

On the basis of their general cultural appearance on common laboratory media the isolates were divided into three groups. These will be referred to subsequently as types A, B, and C (Fig. 1).

The type A isolates were differentiated by their relatively slow-growing compact colonies, with mycelium mostly appressed to the substratum. This group is also characterized by stromalike bodies (Fig. 2) produced in abundance by all isolates. These flattened structures develop at the surface of agar cultures and are often arranged in concentric rings. Similar structures are produced in contact with the glass in flasks of soil media.

Type B isolates were distinguished from those of type A by their production of abundant, fluffy aerial mycelium, particularly in flasks of soil media. The colonies generally grow more rapidly than those of type A. Stromata are not produced under normal cultural conditions.

Type C is a heterogenous group which was differentiated to cover extremely fast-growing isolates. These isolates were invariably obtained from superficial, apparently saprophytic mycelial growth found on a wide variety of grasses and other plants in early spring. On agar the colonies generally grow much more rapidly than those of type B and produce less aerial mycelium. Stromata are produced by certain isolates of this type, e.g. W17. All of these rapidly growing isolates appear to be highly compatible with other isolates. Unlike type A and B isolates, no zone is formed between adjoining colonies.

Two isolates of each type were selected for comparative study as follows: type A — isolates W1 and W10; type B — isolates W2 and W5; type C — isolates W14 and W17.

Influence of Temperature on Growth

The growth response of the six representative isolates of the low-temperature basidiomycete was studied on Difco potato dextrose agar in tubes of the kind described by Ryan *et al.* (14). A uniform disk cut from an agar plate culture was transferred to the medium in one end of each of four tubes for each isolate and temperature. The tubes were supported in a horizontal position and were incubated for 3 weeks at temperatures of 0°, 5°, 10°, 12.5°, 15°, 17.5°, 20°, and 25° C. Temperatures were closely maintained in thermostatically regulated cabinets placed in controlled-temperature rooms. Growth in each tube was measured in millimeters at weekly intervals.

Types A, B, and C differ in their response to temperature, as shown by the results of three experiments with two isolates of each type (Table I). Growth of type A (W1 and W10) was relatively slow at all temperatures, and especially

at 25° C. One isolate of type B (W5) produced significantly more growth than those of type A at all temperatures and W2 also grew more rapidly at 0°, 5°, 20°, and 25° C. Isolate W14 of type C grew much more rapidly than the type A and B isolates at all temperatures. Isolate W17 also grew very rapidly at 5° to 17.5° C but was comparable to the B isolates at the temperature extremes of 0°, 20°, and 25° C.

TABLE I

Effect of temperature on the growth of six isolates of the low-temperature basidiomycete on Difco potato dextrose agar

	Growth in millimeters*					
	Type A		Type B		Type C	
C	W1	W10	W2	W5	W14	W17
0°	7.4	8.6	11.9	14.6	18.0	14.5
5°	22.7	22.5	30.7	28.7	38.6	37.6
10°	32.8	32.9	34.3	37.0	56.1	59.4
12.5°	38.2	37.5	38.4	42.6	65.4	64.1
15°	34.9	34.3	34.6	40.6	66.6	62.3
17.5°	33.5	33.9	36.5	43.6	69.0	57.5
20°	17.0	17.3	25.3	24.8	42.5	21.3
25°	4.5	3.7	9.1	7.6	13.5	8.3

*Average growth in four replicate tubes after incubation for 27 days.

Best growth of all types and isolates studied occurred at 12.5° to 17.5° C. With type B isolates there were no significant differences in growth within this temperature range. All type A isolates tested grew best at 12.5° C. W14 produced significantly more growth at 17° than at 12.5° C and the reverse occurred in all tests with W17.

Type A isolates responded most uniformly to temperature. Type B showed more variability and the isolates classed as type C differed markedly in their response, as in other characteristics. W17 produced only about one-half the growth of W14 at 20° and 25° C.

Influence of Hydrogen-ion Concentration on Growth

The effect of hydrogen-ion concentration on growth of the six isolates of the low-temperature basidiomycete was determined on 'M.Y.G.' agar adjusted to the pH values of 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 8.0, and 9.0. This medium was composed of malt extract (Difco) 5.0 g, yeast extract (Difco) 5.0 g, D-glucose 15.0 g, agar (Difco-Bacto) 17.0 g, and distilled water 1 liter. It was made up with half the required volume of water and, after sterilization, the amount of sterile *N* KOH or *N* HCl needed to adjust a molten sample to the various pH values was determined. Appropriate volumes of the remainder of the medium were then adjusted to the required pH values with calculated amounts of sterile *N* KOH or *N* HCl and sterile distilled water was added to make up the total volume. Equal volumes (15 ml) were transferred to Petri dishes by means of a sterile syringe. After the medium had solidified, the plates were inoculated in the center with a uniform disk cut from an agar plate culture and incubated at 15° C in a controlled-temperature cabinet. Growth, as the

TABLE II

Effect of pH on the growth of six isolates of the low-temperature basidiomycete on M.Y.G. agar

	Growth in millimeters*					
	Type A		Type B		Type C	
pH	W1	W10	W2	W5	W14	W17
3.0	0	0	15.0	11.5	0	0
4.0	27.5	19.5	34.3	32.0	51.3	34.8
5.0	43.8	30.0	44.5	44.5	75.8	67.8
6.0	47.0	36.0	44.5	52.5	83.8	79.8
6.5	45.3	35.5	45.3	47.5	83.8	80.0
7.0	44.8	36.5	46.5	47.0	84.5	81.3
8.0	31.0	28.0	43.0	40.8	75.8	67.5
9.0	15.8	15.8	36.5	31.3	51.3	40.0

*Mean diameters of four replicate cultures after 9 days' growth.

diameter of the colony, was measured in millimeters daily and the average values from four replicate cultures after 9 days' growth are given in Table II.

The pH optima for growth of the six isolates were generally similar in that they lay between pH 6.0 and 7.0. The optimum for the type C isolates was pH 7.0, that of the other isolates was either pH 6.0 or 7.0, but differences in growth over this range were small. The type B isolates were the most tolerant of low pH values, being distinct from those of the other types in their ability to grow at pH 3.0. Growth of the type B isolates, and particularly W2, was also least reduced at the higher pH values. Growth of W2 at pH 9.0 for example, was still approximately 80% of that at the optimum pH. The type A isolates did not grow at pH 3.0 and growth at pH 9.0 was relatively less than that of the other isolates. With both type A isolates there was a lag of 2 days before growth commenced at pH 4.0 and pH 9.0. The type C isolates were similar to type A in failing to grow at pH 3.0, but at all other pH levels their growth rate was conspicuously higher than that of the other types.

Inhibition of Growth by Antibiotics

A comparison was made of the tolerance of the six isolates to the antibiotics acti-dione, filipin, griseofulvin, and patulin. These were incorporated in M.Y.G. agar to give concentrations of 0, 0.1, 1.0, 5.0, 10.0, 50.0, and 100.0 p.p.m. The procedure used was similar to that described for the pH experiments and growth was measured in millimeters at daily intervals. In Table III the results have been summarized as the percentage inhibition of growth at the concentrations indicated. Acti-dione was by far the most toxic of the four antibiotics, and W17 was the only isolate to grow at a concentration above 10 p.p.m. This isolate commenced growth after a lag of 6 days with 50 p.p.m. acti-dione and continued to grow slowly at this concentration until the termination of the experiment. Differences in the percentage inhibition of the three isolate types occurred with all antibiotics and the responses of the representatives of each type were usually very similar. Acti-dione inhibited type A and type B to a similar degree while type C showed rather more resistance. Type C isolates were, however, more strongly inhibited than type

TABLE III

Inhibition by antibiotics of the growth of six isolates of the low-temperature basidiomycete on M.Y.G. agar

Antibiotic	Inhibition, %					
	Type A		Type B		Type C	
	W1	W10	W2	W5	W14	W17
Acti-dione, 10 p.p.m.	50	45	45	50	40	30
Filipin, 100 p.p.m.	25	25	15	15	45	50
Griseofulvin, 100 p.p.m.	40	30	35	35	50	80
Patulin, 100 p.p.m.	15	15	10	10	25	20

A or type B by the other antibiotics; griseofulvin, for example, was particularly toxic to W17 and patulin caused a reduction in growth of W14 and W17 approximately twice that of the other isolates. With the exception of acti-dione, type B isolates were less sensitive to the antibiotics than were those of type A.

HCN Production

The production of HCN by the low-temperature basidiomycete has already been referred to and the following experiments were carried out to compare HCN production by the six isolates under study.

Qualitative tests for detecting HCN released in laboratory culture were made by the following method: 10 ml of a sodium picrate solution (25 g sodium carbonate and 5 g picric acid in 1 liter distilled water) were added to a 6-cm Petri dish which was placed centrally within a 10-cm Petri dish to form a center well; an isolate of the low-temperature basidiomycete was grown in the outer ring, surrounding the small dish, on a medium composed of a 10:1 mixture of sterile soil and soybean meal (Fig. 3). The medium in the control dishes was not inoculated. The HCN released by the fungus in the outer ring diffused into the center well where it reduced the sodium picrate (yellow) to sodium picramate (red).

Tested by this method only the type B isolates gave a positive reaction for HCN production. The dark red color that developed in the center well of dishes inoculated with these isolates is illustrated in Fig. 3, where there is a sharp contrast with the lack of color change in the control and in the dishes inoculated with the A and C types.

The ability of the three isolate types to release HCN under field conditions was compared by making cyanide determinations on infected tissues from alfalfa plants. First-year plants of *Medicago sativa* were inoculated in the fall using the procedure described by Cormack (5), and determinations of HCN content in the crown buds were made in the early spring by the method used by Lebeau and Dickson (12). Each of four random plots was inoculated with one of the isolates and four control plots were left uninoculated. In addition to HCN determinations on the crown buds, survival ratings were made on the plants after suitable re-growth in the shoots had occurred.

The results reported in Table IV show that the type A isolates produce

much higher quantities of HCN in the tissues of *M. sativa* than the type B isolates. No evidence of HCN production by type C isolates was obtained either in the laboratory or under field conditions. Survival appeared to be inversely related to concentration of HCN recovered from the crown bud tissues.

TABLE IV

Production of HCN in crown buds and survival of *M. sativa* infected by different isolates of the low-temperature basidiomycete

Isolate		Concentrations of HCN in crown buds* (p.p.m.)	Average survival (%)
Type	Number		
A	W1	600	18
	W10	665	3
B	W2	175	95
	W5	240	85
C	W14	0	100
	W17	0	100
Control		0	100

*HCN concentrations expressed on dry weight basis of crown bud tissues.

Tolerance of HCN

The production of large quantities of HCN either in culture or in infected plants suggests that the low-temperature basidiomycete may be able to tolerate this substance in high concentrations. Accordingly, growth of the six representative isolates was compared on M.Y.G. agar in which KCN was incorporated at concentrations equivalent to 0, 10, 50, 100, 250, and 500 p.p.m. The procedure was similar to that already described for the pH experiments. After inoculation the Petri dishes were sealed with a broad rubber band to reduce loss of gaseous HCN. The diameters of the colonies were measured after 7 days' incubation at 15° C and the mean values for four replicates of each isolate are given in Table V.

Growth of type A and type C isolates was completely inhibited by approximately one fifth the concentration of HCN that was required for type B. Isolates of types A and C failed to grow at concentrations higher than 50 p.p.m., although the type A isolates were relatively less inhibited than the type C isolates at lower concentrations. Inhibition of growth that occurred was presumably due to the toxicity of the cyanide radical. Addition of KCN to the medium increased the pH to the values shown. However, reference to

FIG. 1. Appearance of cultures of the three types growing on M.Y.G. agar (type A, W1; type B, W2, type C, W14).

FIG. 2. Production of stromata in cultures of W1 (type A) on M.Y.G. agar.

FIG. 3. Petri dishes containing sodium picrate in center well with A-, B-, and C-types of the low-temperature basidiomycete growing on a 10:1 soil – soybean meal mixture in the outer ring. The color change from yellow to dark red has occurred in the center well of the plates inoculated with the B-type indicating a positive test for HCN. Uninoculated Petri dishes on extreme left.

FIG. 4. Isolations made in the spring from plots of *D. glomerata* (upper row) and *M. sativa* (lower row) that were inoculated the previous fall with the B-type of the low-temperature basidiomycete.

PLATE I

Ward *et al.*—Can. J. Botany

TABLE V

Effect of HCN* on the growth of six isolates of the low-temperature basidiomycete on M.Y.G. agar

HCN (p.p.m.)	pH	Growth in millimeters†					
		Type A		Type B		Type C	
		W1	W10	W2	W5	W14	W17
0	6.35	31.5	29.5	40.0	43.0	59.0	60.5
10	6.46	28.5	28.5	39.5	44.0	42.0	49.0
50	6.65	9.0	15.0	39.5	42.0	17.0	10.0
100	7.10	0	0	28.5	32.0	0	0
250	7.55	0	0	11.0	13.0	0	0
500	8.30	0	0	0	0	0	0

*HCN added as KCN.
†Mean diameter of four replicate cultures after 7 days' growth.

Table II indicates that only at concentrations of 250 p.p.m. and higher was the pH change sufficient to have an appreciable effect on growth of any of the isolates.

Pathogenicity

Disease surveys in Western Canada have shown varying degrees of damage in forage crops and turf grass caused by the low-temperature basidiomycete. Much of this variability was caused by fluctuations in environmental conditions affecting disease development. Recent studies, however, indicate that differential infection of the same host may also be caused by variation in virulence of the isolates or strains of this organism. Thus, the data in Table IV show that the A-type isolates are much more pathogenic on *M. sativa* than are the B-type. The same isolates were tested for pathogenicity in temperature-controlled cabinets by the method described by Cormack and Lebeau (7). The following survival percentages of *M. sativa* inoculated under artificial conditions with the three isolate types confirm the previous results obtained from experiments in the field:

Type A	W1	28
	W10	38
Type B	W2	68
	W5	81
Type C	W14	98
	W17	98

Control (uninoculated) 96

In order to compare the relative pathogenicity of these isolates on both grass and alfalfa, tests were conducted in the field on *Dactylis glomerata* and *M. sativa*. Representative isolates of the three types of the fungus were used to inoculate three replications of paired plots of *D. glomerata* and *M. sativa*. The survival percentages of the two host species are recorded in Table VI.

The results show that the type A isolates were much more pathogenic on alfalfa than were the type B isolates. The latter, however, were the most pathogenic on grass. A further indication of the preference of the type B isolates

TABLE VI

Survival of *M. sativa* and *D. glomerata* inoculated with representative isolates of the three types of the low-temperature basidiomycete

Isolate		Average survival (%)	
Type	Number	*M. sativa*	*D. glomerata*
A	W1	0	70
	W10	3	62
B	W2	47	42
	W5	60	33
C	W14	100	100
	W17	100	100
Control		100	100

for this host was obtained by plating out tissue from alfalfa and grass plants infected with these isolates. Twice the number of type B colonies developed in the isolations made from *D. glomerata* tissue as from *M. sativa* tissue. Typical isolation plates are illustrated in Fig. 4. Conversely, more than twice as many type A colonies were obtained from *M. sativa* tissue as from *D. glomerata* tissue when plants infected with type A isolate were used.

Discussion

The results of the experiments described here support the conclusion that the isolates of the low-temperature basidiomycete are of three distinct types. They possess certain features in common, of which their growth in the form of white sterile mycelium bearing basidiomycetous clamp connections is probably the most obvious. The temperature ranges and optima for growth of the isolates were generally similar and below those most commonly found for plant pathogenic fungi (3). The ability of all isolates to make appreciable growth at 0° and 5° C is consistent with their natural activity under snow. The three types could be distinguished by differences in growth rate and in their response to all the other experimental factors to which they were subjected.

The view that the type C isolates are saprophytic, spreading superficially over the surface of vegetation in early spring, finds confirmation in the results reported here. Failure to bring about infection and the absence of HCN production under any conditions indicates lack of pathogenicity, especially of the kind displayed by the type A and B isolates. The high growth rate of the type C isolates may also be a saprophytic characteristic as suggested by Garrett (8) for soil saprophytes. The relative sensitivity of these isolates to antibiotics which might indicate poor competitive saprophytic ability, sensu Garrett, would undoubtedly be offset by their comparatively high growth rate at temperatures where competition is probably greatly reduced.

The differences that were found between the A and the B types are particularly interesting. Both groups are parasitic and exhibit the same peculiar type of parasitism, involving the production of HCN. The type B isolates, however, displayed greater vigor under most cultural conditions. They possessed a somewhat higher growth rate than the type A isolates. In fact,

the method used here to measure growth did not take account of the development of abundant aerial mycelium and the greater colony density characteristic of this type. They grew relatively faster than the type A isolates at the extremes of the temperature range employed and tolerated a wider range of hydrogen-ion concentration. They were also less sensitive to antibiotics and to HCN. The most striking differences between the A and B types lay in production and tolerance of HCN, and in pathogenicity. Type B isolates produced HCN both in laboratory culture and in infected host tissue. No HCN was detected in cultures of the A type but large amounts were produced in infected plants. The type B isolates were far more tolerant of HCN added to the culture medium than were the other isolates and this suggests a correlation between ability to produce HCN in culture and high tolerance. In view of the universal toxicity of this compound the behavior of these isolates is of considerable interest. However, the production of large amounts of HCN in host tissue infected with type A isolates (Table IV) suggests that under these conditions they, too, possess considerable resistance to this substance.

Type A isolates were extremely virulent on the alfalfa species whereas type B isolates were only moderately pathogenic. However, the latter were equal or greater in pathogenicity on the grass species. The type C isolates were not pathogenic on either host. The correlation between severe killing and the recovery of high HCN concentrations from the crown buds of *M. sativa* (Table IV) is emphasized by the results. Survival of alfalfa plants was drastically reduced when the HCN concentration reached 600 p.p.m. in crown buds infected by type A isolates. A much smaller HCN concentration accumulated in plants infected with type B isolates and survival was correspondingly much greater. No HCN was detected in plant tissues inoculated with type C isolates and no pathogenesis occurred.

It may be concluded that the isolates grouped together as the low-temperature basidiomycete include a variety of forms active at below normal temperatures. They have been conveniently classed into three main types, of which two, A and B, are parasitic and the third, C, saprophytic. Of the parasitic groups the A type is possibly the most specialized, producing HCN only in association with the living host and being less tolerant of a number of other limiting factors than the B type.

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References

1. Broadfoot, W. C. 1936. Experiments on the chemical control of snow-mould of turf in Alberta. Sci. Agr. **16**, 615–618.
2. Broadfoot, W. C. and Cormack, M. W. 1941. A low-temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopathology, **31**, 1058–1059.
3. Cochrane, V. W. 1958. Physiology of fungi. John Wiley & Sons, Inc., New York.
4. Cormack, M. W. 1942. Varietal resistance of alfalfa and sweet clover to root- and crown-rotting fungi in Alberta. Sci. Agr. **22**, 775–786.
5. Cormack, M. W. 1948. Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. I. Occurrence, parasitism, and spread of the pathogen. Can. J. Research, C, **26**, 71–85.

6. CORMACK, M. W. 1952. Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. II. Field studies on host and varietal resistance and other factors related to control. Can. J. Botany, **30**, 537–548.
7. CORMACK, M. W. and LEBEAU, J. B. 1959. Snow mold infection of alfalfa, grasses, and winter wheat by several fungi under artificial conditions. Can. J. Botany, **37**, 685–693.
8. GARRETT, S. D. 1956. Biology of root-infecting fungi. Cambridge University Press, London.
9. LEBEAU, J. B. and CORMACK, M. W. 1956. A simple method for identifying snow mold damage on turf grasses. Phytopathology, **46**, 298.
10. LEBEAU, J. B., CORMACK, M. W., and MOFFATT, J. E. 1959. Measuring pathogenesis by the amount of toxic substance produced in alfalfa by a snow mold fungus. Phytopathology, **49**, 303–305.
11. LEBEAU, J. B. and DICKSON, J. G. 1953. Preliminary report on production of hydrogen cyanide by a snow-mold pathogen. Phytopathology, **43**, 581–582.
12. LEBEAU, J. B. and DICKSON, J. G. 1955. Physiology and nature of disease development in winter crown rot of alfalfa. Phytopathology, **45**, 667–673.
13. LEBEAU, J. B. and LOGSDON, C. E. 1958. Snow mold of forage crops in Alaska and Yukon. Phytopathology, **48**, 148–150.
14. RYAN, F. J., BEADLE, G. W., and TATUM, E. L. 1943. The tube method of measuring the growth rate of *Neurospora*. Am. J. Botany, **30**, 784–799.

NORTHERN PYRENOMYCETES

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Abstract

Two collecting trips in Gaspesian Park, Quebec, during the summer of 1957, yielded a considerable number of pyrenomycetes. In this paper records of 87 species or varieties in 46 genera are presented. Two new species, *Linocarpon umbelliferarum* Barr and *Pseudomassaria foliicola* Barr, are described. Five previously described species are transferred to other genera, and the following new combinations are proposed: *Gibbera cassandrae* (Peck) Barr, *Gibbera grumiformis* (Karsten) Barr, *Gibbera kalmiae* (Peck) Barr, *Gibberidea kalmiae* (Peck) Barr, and *Limacinia arctica* (Woronichin) Barr.

Introduction

The Gaspé region of the province of Quebec has an interesting phanerogamic flora from which to study the occurrence of pyrenomycetes. The scarcity of records of any fungi provided an impetus to collect and study in this region. In 1957, over 250 collections were made during two periods, from July 6 to July 12 and from August 18 to August 22. The following pyrenomycetes are reported as a contribution to knowledge of their distribution. Where elucidation seems necessary, notes and brief descriptions are appended. Two new species are described. Several other species appear to be previously unknown, but further collections are needed before their relationships can be correctly determined.

The collecting areas visited in the park include the woods, streams, and trails in the vicinity of the chalet, the top of Mont Albert, and two lakeshores within a few miles of the chalet. Scoggan (1950) has discussed the physical features, climate, glaciation, and life form classification of the region. The host plants cited in the following list of fungi are determined in accordance with his systematic arrangement.

The necessary funds for the field work were provided by the National Research Council of Canada, for field expenses under my Postdoctorate Fellowship. I wish to express my appreciation to the Council for making these collecting trips possible. I am grateful to Mr. L. P. Gagnon, General Superintendent of Parks and Reserves, Département de la Chasse et de la Pêche, of the Province of Quebec, for permission to collect in Gaspesian Park. Mlle. Laurette Gagné, Le Gîte du Mont Albert, was most helpful in arranging for guides within the Park, and in making accommodations for drying the fungi. The plant specimens were identified by Dr. R. B. Livingston of the Department of Botany of the University of Massachusetts. For the loan of type specimens to compare with my collections, I am indebted to Mr. S. J. Smith, Senior Curator of Botany at the New York State Museum, Albany, New York, and

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A complete set of identified specimens and type material of the two new species are deposited in the Mycological Herbarium, Plant Research Institute, Central Experimental Farm, Ottawa (DAOM).

As in the first paper in this series (Barr 1959), the fungi are cited in alphabetical order within the family.

Taxonomy

Pseudosphaeriaceae

Wettsteinina macrotheca (Rostrup) Mueller, Sydowia, 12: 203. 1958.
On *Eriophorum angustifolium* Roth, *1956*.

This collection is similar to those reported on *Carex* from northern Quebec (Barr 1959). The spores reach a length of 48 μ, somewhat larger than the size range found previously, but are identical in shape.

Wettsteinina mirabilis (Niessl) von Hoehnel, Sitz. K. Akad. Wiss. Wien Math.-nat. Kl. 116: 635. 1907.
On *Artemisia borealis* Pall., *2266*, *Coptis trifolia* (L.) Salisb. var. *groenlandica* (Oeder) Fassett, *1929B*.

The fungus on *Coptis* is immature and has smaller spores than is generally found in this species. In the collection on *Artemisia*, the spores frequently have three secondary septa, one above and two below the primary septum. After discharge from the ascus the spores become brown and the wall roughened.

Wettsteinina niesslii Mueller, Sydowia, 4: 204. 1950 (Fig. 1).
On *Eriophorum angustifolium* Roth, *1956*.

This species occurred on the same collection of *Eriophorum* as did *W. macrotheca*. The two species are readily distinguished microscopically. *W. niesslii* has long fusoid spores, 52–67×4.5–6 μ, whereas the spores of *W. macrotheca* are ellipsoid to cylindrical, 31.5–48×7.5–12 μ. *W. niesslii* is described by Mueller (1950) as having three- to five-septate spores. My collection from Mont Albert has spores with only one central septum. However, it agrees well with Mueller's description in other respects. A collection on *Carex* from Great Bear Lake, Northwest Territories, is more mature than either Mueller's or my collections. The spores are 52–63×10–14 μ, light yellowish, and finally six-to seven- (rarely eight-) septate. The gelatinous coating surrounding the spore is 3–4.5 μ wide.

According to the description of *Sphaerella bacillifera* Karsten (1883), it is close to *W. niesslii*. The spores are described as uniseptate, greenish hyaline, and 45–60×6 μ. Munk (1957) transferred the species to *Leptosphaeria* and reported the spores as 36–45×4–5 μ, with a primary central septum and two to four secondary septa.

Dothideaceae

Mycosphaerella caulicola (Karsten) Lind, Danish Fungi, p. 208. 1913.
On *Geum canadense* Jacq., *2022*; *Hieracium pratense* Tausch., *1908*.

These collections are identical with Munk's (1957) concept of the species.

The depressed, grouped ascostromata, narrow asci, and spores are distinctive. *M. caulicola* has been reported on a number of herbaceous plants in Europe.

Mycosphaerella colorata (Peck) House, N.Y. St. Mus. Bull. 233–234: 26. 1921 (Fig. 2).

On *Kalmia angustifolia* L., *2011*, *2216*.

Type material of *Sphaerella colorata* Peck is identical with these collections.

Mycosphaerella coptis (Schw.) House, N.Y. St. Mus. Bull. 233–234: 27. 1921.

On *Coptis trifolia* (L.) Salisb. var. *groenlandica* (Oeder) Fassett, *1929A*, *1975*.

Pycnidia of *Septoria coptidis* B. & C. are erumpent through the upper surface of leaf spots, and the *Mycosphaerella* develops in the same spots in the lower side of the leaf.

Mycosphaerella filicum (Desm.) Starb., Bihang till K. Svenska Vetensk.-Akad. Handl. 15, Afd. III(2): 9. 1889.

On *Cystopteris fragilis* (L.) Bernh., *2000*.

Mycosphaerella lineolata (Rob. *in* Desm.) Schroeter, Die Pilze Schlesiens, 2: 339. 1894.

On *Carex* sp., *1966*, *2276*; *Deschampsia caespitosa* (L.) Beauv. var. *littoralis* (Reut.) Richter, *1962*; *Phleum pratense* L., *2005*.

Mycosphaerella lycopodii (Peck) House, N.Y. St. Mus. Bull. 233–234: 28. 1921.

On *Lycopodium annotinum* L. var. *acrifolium* Fernald, *1959B*; *Lycopodium annotinum* L. var. *pungens* (La Pyliae) Desv., *1959A*.

Mycosphaerella maculiformis (Fr.) Schroeter, Die Pilze Schlesiens, 2: 333. 1894.

On *Betula papyrifera* Marsh., *1949*; *Pyrus americana* (Marsh.) DC., *2038*; *Salix* sp., *2040*, *2267*.

Mycosphaerella minor (Karsten) Johans., Öfversigt af K. Vetensk.-Akad. Förh. 1884(9): 165. 1884.

On *Andromeda glaucophylla* Link, *2205*; *Armeria maritima* (Mill.) Willd. var. *labradorica* (Wallr.) Lawr., *2268*; *Artemisia borealis* Pall., *2266*; *Epilobium angustifolium* L., *1911*; *Lonicera canadensis* Marsh., *2003*; *Osmunda regalis* L. var. *spectabilis* (Willd.) Gray, *1937*; *Pyrola secunda* L., *1907*; *Vaccinium ovalifolium* Sm., *1952*.

Mycosphaerella tassiana (de Not.) Johans., Öfversigt af K. Vetensk.-Akad. Förh. 1884(9): 167. 1884. var. *tassiana*.

On *Arenaria obtusiloba* (Rydb.) Fern., *2269*; *Carex* sp., *1958*; *Empetrum nigrum* L., *1970*; *Ranunculus acris* L., *2001*.

Mycosphaerella tassiana (de Not.) Johans. var. *arctica* (Rostr.) Barr, Contrib. Inst. Bot. Univ. Montreal, 73: 24. 1959.

On *Sisyrinchium montanum* Greene, *2023*.

Mycosphaerella tassiana (de Not.) Johans. var. *arthopyrenioides* (Auersw.) Barr, Contrib. Inst. Bot. Univ. Montreal, 73: 26. 1959.

On *Plantago major* L., *1892*; *Pyrola secunda* L., *1907*.

Mycosphaerella vaccinii (Cke.) Schroeter, Die Pilze Schlesiens 2: 335. 1894.
On *Andromeda glaucophylla* Link, *1982*.

Although occurring on leaves of *Andromeda* rather than *Vaccinium*, this collection cannot be distinguished from others of *M. vaccinii*. The ascostromata are grouped and hypophyllous, the asci numerous and narrow, and the spores are 16.5–22.5×2–3 μ, fusoid and curved.

CAPNODIACEAE

Limacinia arctica (Woronichin) comb. nov. (Figs. 3, 4).
Aithalomyces arctica Woronichin, Ann. Mycol. 24: 149. 1926.

Ascostromata 150–265 μ in diameter, globose, superficial on coarse black subiculum, hyphae dark brown, short-celled, constricted at the septa, thick-walled, 13.5–18 μ wide, walls roughened, branched, wall of ascostroma thin, hyphae appendages dark brown, septate, not or slightly constricted, anastomosing with the subiculum. Asci 72–100×33–48 μ, saccate, apex rounded, base rounded and sessile or foot-like, wall double, thickened, fasciculate, aparaphysate. Spores 33–39×12–18 μ, yellowish brown to olive-brown, ellipsoid-fusoid, ends narrowed, rounded or pointed, straight, three-septate, not constricted until fully mature, wall double, thick, smooth, contents guttulate, crowded in the ascus.

On branches of *Empetrum nigrum* L., *2270*.

This collection agrees in all respects with Woronichin's (1926) description of *Aithalomyces arctica*. The species was described from Russian Lapland on *Arctostaphylos uva-ursi*, *Empetrum nigrum*, and *Phyllodoce taxifolia*. As far as I can determine, the genus *Aithalomyces*, of which *A. arctica* is the type species, cannot be distinguished from *Limacinia*. Woronichin stressed the lack of an ostiole in *Aithalomyces*, but as discussed previously (Barr 1955), the pore of ascostromata of this type is generally small and indistinct. *Aithalomyces rhododendri* Woronichin (1926), the only other species described in this genus, probably should be considered a species of *Limacinia* as well. It is said to differ from *A. arctica* in forming a thick cushion-like subiculum and in shorter and narrower spores.

L. arctica is closely related to *L. alaskensis* Sacc. and Scalia. The chief differences between the two lie in the smaller ascostromata, narrower asci, and smaller spores of the latter species. In growth habit and mycelial appendages, they are similar.

PLEOSPORACEAE

Dendropleella hirta (Fr.) Munk, Dansk Bot. Arkiv. 15(2): 126. 1953.
On branches of *Sambucus pubens* Michx., *2212*.

This collection agrees in all respects with Munk's (1957) description of the species.

Didymella glacialis Rehm, Hedwigia, 21:121. 1882.
On *Carex* sp., *2254*.

D. glacialis was described on grass from the Tyrol. It has been reported on *Carex* and *Juncus* as well as grasses from Spitzbergen, Norway, Siberia, and various localities in the Alps. The papillate ascostromata, numerous narrow

asci, and fusoid, 13.5–16.5×3–4 μ spores distinguish this species from others occurring on *Carex*.

Gibberidea kalmiae (Peck) comb. nov. (Figs. 5–7).

Leptosphaeria kalmiae Peck, 39th Rept. N.Y. State Mus. p. 53. 1886.

Ascostromata 385–460 μ in diameter, 300–370 μ high, globose to depressed, immersed in wood in a single row, erumpent through elongate slits in the bark, black, short papillate, wall thick, 82–130 μ wide, blackened externally, bases immersed in and connected by blackened stromatic hyphae. Asci 97–127× 7.5–9 μ, cylindrical, apex rounded, base narrowed to a short stalk, wall double, thickened above, numerous, pseudoparaphysate. Spores 16.5–20×5–7 μ, yellowish brown, ellipsoid, ends narrowed and rounded, straight to inequilateral, three-septate, deeply constricted at the central septum, wall thick, double, smooth, contents guttulate, uniseriate in the ascus.

On branches of *Kalmia angustifolia* L., *2203*; C. H. Peck, Grassy Pond, Adirondack Mts., New York, June, 1885 (*type* of *Leptosphaeria kalmiae*).

The collection from Mont Albert agrees in all respects with Peck's type of *Leptosphaeria kalmiae*. This fungus, with thick-walled, grouped ascostromata, united at the bases and growing on woody branches, is typical of the genus *Gibberidea* rather than of *Leptosphaeria*. *G. kalmiae* appears to be close to *G. rhododendri* (Niessl) Petrak, according to description. I have seen no material of the latter to determine the specific differences.

Leptosphaeria doliolum (Fr.) Ces. & de Not., Comment. Soc. Critt. Ital. 1: 234. 1863.

On *Achillea millefolium* L., *1997*; *Anaphalis margaritacea* (L.) B. & H., *2249*; *Aster* sp., *2253*; *Epilobium glandulosum* Lehm. var. *adenocaulon* (Haussk.) Fernald, *2250*; *Heracleum lanatum* Michx., *2198*; *Hieracium pratense* Tausch., *2252*; *Potentilla norvegica* L., *2246*.

Leptosphaeria hyperborea (Fckl.) Berl. & Vogl., Addit. Syll. Fung. p. 140. 1886.

On *Arctostaphylos alpina* (L.) Spreng., *1969*; *Armeria maritima* (Mill.) Willd. var. *labradorica* (Wallr.) Lawr., *1957*.

For the present, *L. hyperborea* is retained in the genus *Leptosphaeria*. It actually has more affinities with members of the genus *Phaeosphaeria*. However, it is also closely related to *Leptosphaerulina* in the Pseudosphaeriaceae. *Leptosphaerulina pulchra* (Wnt.) Barr differs from *Leptosphaeria hyperborea* chiefly in somewhat broader spores which are three- to five- (rarely six-) septate with one vertical septum. In growth habit and in the shape of all structures there is a marked similarity.

Leptosphaeria lycopodina (Mont.) Sacc., Syll. Fung. 2: 81. 1883.

On *Lycopodium annotinum* L. var. *pungens* (La Pyliae) Desv., *1959A*, *1978*.

This species and the following are related but readily separable. *L. lycopodina* produces copious dark-brown mycelium and has larger straight spores, 27–33× 9–12 μ, than does *L. marciensis*. The latter species is glabrous or nearly so, with straight to curved spores which measure 24–27×6–7 μ. Holm (1957) distinguishes these two species on the above characteristics.

Leptosphaeria marciensis (Peck) Sacc., Syll. Fung. 2: 80. 1883.
On *Lycopodium annotinum* L. var. *pungens* (La Pyliae) Desv., *1959A*, *1978*; *L. annotinum* L. var. *acrifolium* Fernald, *1959B*.

Leptosphaeria ogilviensis (Berk. & Br.) Ces. & de Not., Comment. Soc. Critt. Ital. 1: 235. 1863.
On *Anaphalis* sp., *1909*; *Solidago* sp., *2179*.

Melanomma pulvis-pyrius (Fr.) Fckl., Symb. Mycol. p. 160. 1870.
On *Pyrus americana* (Marsh.) DC., *1939*; rotting twigs, *2232*.

Nodulosphaeria aquilana (D.Sacc.) Holm, Symbolae Bot. Upsalienses 14(3): 83. 1957.
On *Hieracium pratense* Tausch., *1908*.

This collection is identical in all respects with Holm's (1957) description of the species.

Nodulosphaeria modesta (Desm.) Munk, Dansk Bot. Ark. 15(2): 136. 1953.
On *Heracleum lanatum* Michx., *1951*; *Ranunculus acris* L., *2001*; Compositae stalks, *1950*.

N. modesta, according to Holm (1957) is common on numerous host plants. These collections agree with his concept of the species.

Ophiobolus erythrosporus (Riess) Winter, *in* Rabenhorst, Kryptogamenfl. 1(II): 525. 1887.
On *Achillea* sp., *1997*; *Aster* sp., *2253*.

Ophiobolus rubellus (Fr.) Sacc., Michelia, 2: 324. 1881.
On *Heracleum lanatum* Michx., *1951*, *2198*.

Phaeosphaeria eustoma (Fckl.) Holm, Symb. Bot. Upsalienses 14(3): 109. 1957.
On *Carex* sp., *2248*; *Deschampsia caespitosa* (L.) Beauv. var. *littoralis* (Reut.) Richter, *1962*.

Phaeosphaeria herpotrichoides (de Not.) Holm, Symb. Bot. Upsalienses 14(3): 115. 1957.
On *Carex* sp., *2020*; *Phleum pratense* L., *1999*, *2005*, *2247*; grass, *1912*

Pleospora ambigua (Berl. & Bres.) Wehmeyer, Mycologia, 43: 42. 1951.
On *Anaphalis margaritacea* (L.) B. & H., *2249*; *Hieracium pratense* Tausch., *2252*.

Pleospora helvetica Niessl, Verhandl. nat. Ver. in Brünn, 15: 191. 1876.
On *Arenaria obtusiloba* (Rydb.) Fern., *1954*; *Campanula rotundifolia* L., *2277*; *Heracleum lanatum* Michx., *1951*; *Solidago* sp., *2179*.

Pleospora moravica (Petrak) Wehmeyer, Amer. Journ. Bot. 39; 241. 1952.
On *Potentilla fruticosa* L., *2271*.

Pleospora phaeospora (Duby) Ces. & de Not., Comment. Soc. Critt. Ital. 1: 44. 1863.
On *Campanula rotundifolia* L., *2176*.

Sporormia leporina Niessl, Oesterr. Bot. Zeitschr. 28: 44, 96. 1878.
On horse dung in old stable, *2222*.

Sporormia minima Auerswald, Hedwigia, 7: 66. 1868.
On moose dung, *2211*.

Strickeria obducens (Fr.) Winter, *in* Rabenhorst, Kryptogamenfl. 1(II): 285. 1887.
On *Alnus crispa* (Ait.) Pursh., *2178A*.

Trichometasphaeria gloeospora (Berk. & Curt.) Holm, Symb. Bot. Upsalienses 14(3): 144. 1957.
On *Aralia nudicaulis* L., *1934*, *1948*; *Hieracium pratense* Tausch., *2252*.

Botryosphaeriaceae

Botryosphaeria abietina (Prill. & Delacr.) von Arx & Mueller, Beitr. Kryptogamenfl. Schweiz, 11(1): 42. 1954 (Fig. 8).
On *Abies balsamea* (L.) Mill., *1936*.

This collection agrees in all respects with von Arx and Mueller's (1954) description of the species. It has not been recorded previously from North America.

Lophiostomataceae

Lophiostoma thujae Ellis & Everhart, North Amer. Pyreno. p. 226. 1892 (Figs. 9, 10).
On *Thuja occidentalis* L., *1927*.

This collection has spores 15–18×4.5–6 μ, which are larger than given in the description (14–16×3 μ). Otherwise it agrees closely with this species, which was described on *Thuja* from New York and Ontario (Ellis and Everhart 1892).

Venturiaceae

Apiosporina morbosa (Schw.) von Arx, Acta Bot. Neerlandica, 3(1): 86. 1954.
On *Prunus pensylvanica* L., *2028*.

Gibbera cassandrae (Peck) comb. nov. (Figs. 11, 12).
Venturia cassandrae Peck, 38th Rept. N.Y. State Mus. p. 104. 1885.

Ascostromata 66–82 μ in diameter, 80–100 μ high, conical, erumpent from beneath scales and superficial on epidermis, amphigenous, loosely scattered on diffuse brown spots, also on petioles and branches, wall thin and brown below, blackened above, setose around the apex, setae few, 48–68 μ long, 6–7.5 μ wide near base, blackish brown, simple, pointed, brown hyphae arising from lower sides and connected with thin hypostroma in leaf tissues. Asci 45–60× 15–21 μ, oblong saccate, ends rounded, sessile or nearly so, wall double, thickened above, pseudoparaphyses few. Spores 18–21×6–9 μ, green becoming olivaceous at maturity, clavate, ends obtusely pointed, straight to inequilateral, one-septate in the middle, slightly constricted, upper cell broader than the lower, wall smooth, contents finely guttulate, crowded in the ascus.

On leaves and branches of *Chamaedaphne calyculata* (L.) Moench., *2199*;

Caroga, Fulton Co., New York, C. H. Peck, July, 1884 (*type* of *Venturia cassandrae*).

The collection from Mont Albert is identical in all respects with type material. The characteristics of erumpent-superficial ascostromata and thin hypostromata are those of the genus *Gibbera* as it is now delimited (Mueller and von Arx, 1950, von Arx 1952, 1954), rather than of *Venturia*. The latter genus is limited to species with immersed ascostromata.

Gibbera cassandrae differs from *G. pulchella* (Cke. & Peck) Petrak, also occurring on *Chamaedaphne*, in several respects. The ascostromata of *G. cassandrae* are smaller and not thickly grouped, the asci are shorter and broader, and the spores decidedly larger. Those of *G. pulchella* measure 9–15.5×(2.5–)3.5–4.5(–5) μ.

Shear (1948) considered *Venturia cassandrae* synonymous with *V. arctostaphyli* Cooke and Harkness. I have not seen authentic material of the latter species, but from the description it differs from *G. cassandrae* in scattered ascostromata and smaller spores. Apparently there is a complex of species or varieties of *Gibbera* on members of the Ericaceae. In numerous collections, I have seen forms which differ one from the other only in relatively minor characteristics of spore size and shape, scattering or grouping of the ascostromata, or amount of subiculum formed. Still further study of these and other collections is required, before a pattern of speciation can be discerned.

Gibbera conferta (Fr.) Pętrak, Sydowia, 1: 200. 1947.
On *Vaccinium* sp., *2191*.

Gibbera dickiei (Berk. & Br.) von Arx, Tijd. over Plantenz. 58: 265. 1952.
On *Linnaea borealis* L. var. *americana* (Forbes) Rehder, *1924*, *1930*.

Gibbera grumiformis (Karsten) comb. nov. (Figs. 13–15).
Sphaerella grumiformis Karsten, Mycol. Fenn. 2: 173. 1873.
Mycosphaerella grumiformis (Karst.) Starb., Bihang till K. Svenska Vet.-Akad. Handl. 16, Afd. III, No. 3, p. 3. 1890.
Phaeosphaerella grumiformis (Karst.) Sacc., Malpighia 10: 270. 1896.

Ascostromata 115–165(–300) μ in diameter, irregularly rounded, black, shining, epiphyllous, erumpent, wall 18–30 μ thick, of coarse brown cells, increasing in thickness toward the base and forming a thick hypostroma which bears a single ascostroma, non-setose. Asci 40–60×12–16.5 μ, oblong saccate, apex rounded, base rounded and sessile or abruptly narrowed to a short foot-like stalk, wall double, thickened above, numerous, pseudoparaphysate. Spores 15–19.5×4.5–6 μ, green becoming olive-brown, broadly clavate, or oblong, ends rounded or lower end obtusely pointed, straight to inequilateral, one-septate in the middle or above it, slightly constricted, upper cell broader than the lower, wall double, finely roughened at maturity, contents minutely guttulate, crowded in the ascus.

On leaves of *Arctostaphylos alpina* (L.) Spreng., *1969*.

This species is distinctive in the dense, hypostromatic base which bears a single non-setose ascostroma. It belongs in the typical subgenus of *Gibbera*, with a well-developed hypostroma. *G. grumiformis* appears to be of scattered

distribution. It has been reported on species of *Arctostaphylos* from Russian Lapland, Siberia, Sweden, Norway, and Novaya Zemlya.

Gibbera kalmiae (Peck) comb. nov. (Figs. 16, 17).

Venturia kalmiae Peck, 28th Rept. N.Y. State Mus. p. 82. 1875.

Coleroa kalmiae (Peck) Rehm, Ann. Mycol. 9: 288. 1911.

Ascostromata 37–115 μ in diameter, globose to conical, erumpent-superficial, grouped on gray or brown leaf spots, or scattered on leaf or branch or dried flower, arising from a thin, subcuticular or subepidermal hypostroma, wall thin, darkened toward apex, pore 13–20 μ wide, surrounded by numerous blackish-brown setae, 23–60 μ long, 5.5–7.5 μ wide at base, pointed, usually curved, septate or simple. Asci 27–57×6.5–13.5 μ, oblong to saccate, apex rounded, base narrowed to a foot-like stalk or rounded and sessile, wall double, thickened above, pseudoparaphyses few. Spores 10–15×3–5 μ, greenish becoming olive-green to olive-brown, fusoid or clavate, narrowed to the obtuse or pointed ends, straight to inequilateral, one-septate in the middle, slightly constricted and becoming deeply constricted at maturity, upper cell broader than the lower, wall double, smooth or finely roughened at maturity, contents guttulate, overlapping biseriate or crowded in the ascus.

On *Kalmia angustifolia* L., *2011*, *2203*, *2216*; *K. polifolia* Wang., *1961*; *K. glauca* (=*polifolia*), Kasoag, Oswego Co., New York, C. H. Peck, July, 1874 (*type* of *Venturia kalmiae*).

The type material of *Venturia kalmiae* Peck is on leaves, thickly grouped, and has spores which are 10–12.5×3–3.5 μ. The collection on *Kalmia polifolia* (*1961*) is similar to the type collection. The collections on *Kalmia angustifolia* and several additional collections from Massachusetts are somewhat better developed, with spores 11.5–15×3–5 μ. When the fungus develops on branches it is more scattered on the thin hypostroma than when it develops on leaf spots. It frequently occurs scattered on the edges of spots caused by *Mycosphaerella colorata* (Peck) House. *Gibbera kalmiae* is a member of the typical subgenus of *Gibbera* by virtue of its erumpent grouped ascostromata on a hypostroma.

Gibbera myrtilli (Cke.) Petrak, Sydowia, 1: 200. 1947.

On *Vaccinium ovalifolium* Sm., *1952*.

Gibbera petrakii Mueller, Sydowia, 8: 61. 1954.

On leaves of *Arctostaphylos alpina* (L.) Spreng., *1969*.

G. petrakii was described from the Swiss and Italian Alps. It is a minute species, with ascostromata 33–80 μ in diameter and spores 9–12×3–3.5 μ, usually with the lower cell bent. The ascostromata are scattered or somewhat grouped on leaves. In the same collection of *Arctostaphylos* there is a closely related fungus with ascostromata 56–115 μ in diameter. The spores are similar in shape to those of *G. petrakii* but measure 15–19.5×3–3.5 μ. It could well be considered a large form or variety of *G. petrakii*.

Phaeocryptopus nudus (Peck) Petrak, Ann. Mycol. 36: 15. 1938.

On *Abies balsamea* (L.) Mill., *1936*.

Rehmiodothis betulina (Fr.) von Arx, Acta Bot. Neerl. 3(1): 84. 1954.
On *Betula glandulosa* Michx., *2260*.

The asci are immature in this collection, but the stromata and minute locules are in accordance with European specimens which I have studied, and with descriptions of the species.

Venturia chlorospora (Ces.) Karst. emend. Aderhold, Hedwigia, 36: 82. 1897.
On *Salix* spp., *1973*, *1981*, *2041*, *2040*, *2267*.

Venturia ditricha (Fr.) Karsten, Mycol. Fenn. 2: 188. 1873.
On *Betula glandulosa* Michx., *1974*, *2274*; *Betula* spp., 1901, 1949.

Venturia fimbriata Dearness & House, Bull. N.Y. State Mus. 233–234: 43. 1921.
On *Achillea millefolium* L., *1997*; *Silene acaulis* L., *2279*.

I have recently described and discussed this fungus (Barr 1959). The collections listed above are similar to type material from New York. The specimens on *Achillea* are fully mature and their spores reach a size of 7.5–9×3.5–4.5 μ.

Venturia grossulariae (Auersw. & Fleisch.) Sacc., Syll. Fung. 1: 589. 1882 (Figs. 18, 19).
On *Ribes* sp., *1925*.

Venturia grossulariae seems to have been collected rarely. There are a few reports of the fungus from Europe but none, to my knowledge, from North America. The ascostromata develop on the under side of fallen leaves. The spores measure 7.5–10×3.5–4.5 μ, with the septum below the middle.

Venturia macularis (Fr.) Mueller & von Arx, Ber. Schweiz. Bot. Gesell. 60: 366. 1950.
On *Populus tremuloides* Michx., *2037*.

Venturia rumicis (Desm.) Winter *in* Rabenhorst, Kryptogamenfl. 1(II): 435. 1887.
On *Rumex acetosella* L., *2004*.

Xenomeris raetica (Mueller) Petrak, Sydowia, 8: 291. 1954 (Fig. 20).
On *Arctostaphylos alpina* (L.) Spreng., *1969*.

In this material the spores measure 10.5–12×3–3.5 μ, whereas Mueller (1954) described them as 7–8×2.5–3 μ. Despite the discrepancy noted, I have no doubt of the identity of this fungus. The ascostromata are small and lack setae. They are seated sparsely on a cushion-shaped hypostroma which is immersed in the leaf tissue.

HERPOTRICHIELLACEAE

Herpotrichiella fusispora Barr, Contrib. Inst. Bot. Univ. Montreal, 73: 28. 1959.
On *Phyllodoce coerulea* (L.) Bab., *1967*, *1971*.

Herpotrichiella polyspora Barr, Contrib. Inst. Bot. Univ. Montreal, 73: 29. 1959.
On *Empetrum nigrum* L., *1970*.

This collection is similar to those on *Cassiope* except for slightly smaller spores measuring 6–9×2–3 μ.

Hysteriaceae

Lophium mytilinum Fries, Syst. Myc. 2: 533. 1823.
On *Abies balsamea* (L.) Mill., *2251.*

Mytilidion decipiens (Karst.) Sacc., Michelia, 1: 55. 1877.
On *Juniperus communis* L. var. *depressa* Pursh., *2194*; *J. communis* var. *montana* Ait., *2202.*

Meliolaceae

Meliola nidulans (Schw.) Cooke, Grevillea, 11: 37. 1882 (Figs. 21–23).
On *Vaccinium vitis-idaea* L., *1932.*

The genus *Meliola* is considered (Luttrell 1951) as a member of the Erysiphales. I do not know enough of this predominantly tropical genus to be able to judge whether this species belongs in it or not. My material has thick-walled asci and broad paraphyses. *M. nidulans* has been reported from the southern United States, but not, so far as I could determine, north of New Jersey.

Sordariaceae

Coniochaeta scatigena (Berk. & Br.) Cain, Univ. Toronto Studies, Biol. Ser. 38: 62. 1934.
On horse dung in old stable, *2240.*

Sordaria pilosa Mouton, Bull. Soc. Bot. Belgique, 25: 144. 1886.
On horse dung in old stable, *2240.*

Sordaria pleiospora Winter, Hedwigia, 10: 161. 1871.
On horse dung in old stable, *2222*, *2223.*

Polystigmataceae

Glomerella cingulata (Stonem.) Spauld & von Schrenk, U.S.D.A. Bur. Pl. Industry Bull. 44: 29. 1903.
On *Sarracenia purpurea* L., *2207.*

Paradidymella tosta (Berk. & Br.) Petrak, Ann. Mycol. 25: 238. 1927.
On *Epilobium angustifolium* L., *1998*, *2255.*

Previously I had included the genus *Paradidymella* in the Diaphorthaceae (Barr 1959). However, the structure of the ascus and perithecium is typical of members of the Polystigmataceae. Munk (1957) has transferred this genus from Diaporthaceae to Polystigmataceae.

Phyllachora kalmiae (Peck) Petrak, Ann. Mycol. 39: 283. 1941.
On *Kalmia polifolia* Wang., *1961*, *2264.*

Both of these collections bear only the black, sterile stromata. Petrak's (1941) description and discussion of *Phyllachora kalmiae* is based upon a specimen collected on Timagami Island, Ontario. I have examined fertile material from Nova Scotia, and found it to agree with Petrak's description.

As Petrak noted, Peck (1873) described the spores of *Dothidella kalmiae* as hyaline, one-septate, and 10–12×5–6 μ. On the other hand, the spores of *Phyllachora kalmiae* are one-celled and measure 13–21(–25)×5–7(–10) μ. It is possible that Peck confused the spores of an imperfect stage with those of the ascus stage. The type of Peck's species bore no asci that I could find, but does have two pycnidial forms. One of these has hyaline conidia, one-septate and 8–11×2–3 μ, borne on short conidiophores in locules within the stroma. It seems logical that the fungus which Peck described is identical with that which Petrak described. The blackened stromatic areas on branches are most distinctive.

Physalospora hyperborea Baeumler, Ann. Natur. Hofmus. Wien p. 439. 1899.
On *Phyllodoce coerulea* (L.) Bab., *1971*, *2272*.

Physalospora vitis-idaeae Rehm, Ann. Mycol. 4: 40. 1906 (Figs. 24–25).
On *Vaccinium vitis-idaea* L., *1972*.

P. vitis-idaeae is similar to *P. vaccinii* (Shear) von Arx and Mueller, which occurs on *Vaccinium macrocarpon*. The latter species is larger in all measurements. *P. vitis-idaeae* was previously known only from Europe. It shows many similarities with *P. hyperborea* on *Phyllodoce* and *Cassiope*, differing by larger perithecia which are often setose around the beak. The spores of *P. vitis-idaeae* are generally broader and are surrounded by a narrow gelatinous coating, in contrast to those of *P. hyperborea*.

Plectosphaera clarae-bonae (Speg.) Theissen, Ann. Mycol. 14: 414. 1916.
On leaves of *Vaccinium vitis-idaea* L., *1932*, *2217*.

P. clarae-bonae is widely distributed in Europe but has not been reported previously from North America. These collections are entirely similar to von Arx and Mueller's (1954) description. A distinctive feature of the species is the cushion-like mass of hyaline, parallel hyphae at the apex of the perithecium.

Pseudomassaria foliicola sp. nov. (Figs. 26–28).

Peritheciis 60–100 μ diametro, globosis, immersis, hypophyllis, erumpentibus; peridio tenui, brunneo, ad apicem nigrescente. Ascis 31–42×7.5–9 μ, oblongo-ellipsoideis, apicis cum poro amyloideo, paraphysatis. Sporis 10.5–13.5×3–4 μ, subflavis, fusoideis vel clavatis, infra medium 1-septatis.

In foliis mortuis *Corni canadensis* L. Specimen typicum legit M. E. Barr, n. *1905*, loc. "Mont Albert, Gaspesian Park, Quebec", Julio 6, 1957.

Perithecia 60–100 μ in diameter, globose, immersed, hypophyllous, thickly scattered, wall thin, about 9 μ wide, cells light brown at sides and base, blackened around the erumpent apex; pore 13–20 μ wide, periphysate, in face view cells contorted and irregular, a few branched brown hyphae arising from sides. Asci 31–42×7.5–9 μ, oblong ellipsoid, narrowed toward base and apex, apex rounded, base short stalked, wall single, thin, slightly thickened above, pore blue in Melzer's solution, paraphyses slender, guttulate, deliquescing. Spores 10.5–13.5×3–4 μ, yellowish, fusoid clavate, broadest at the rounded apex, narrowed to the pointed base, straight or inequilateral, one-septate in the lower third, not constricted, wall thin and smooth, contents homogeneous or minutely guttulate, overlapping obliquely in the ascus.

On overwintered leaves of *Cornus canadensis* L., *1905* (*type*), *1926*.

Three species of *Pseudomassaria* have been described on *Cornus*, but all develop on branches of the woody species, and are distinctly larger than this fungus. *P. foliicola* is characteristic of the genus in structure of perithecia and asci, and in the apiospores. It is similar in many respects to *P. inconspicua* (Johans.) Barr on *Saxifraga*, but differs from that species in size.

Venturia clintonii Peck was described on leaves of *Cornus* and has unequally septate spores. That fungus, however, is a true *Venturia*, and the name is synonymous with *Venturia systema-solare* (Fckl.) Winter (Mueller 1957).

A species of *Septoria*, referable to *Septoria canadensis* Peck, is present on the same leaves as *Pseudomassaria foliicola*. The pycnidia are similar in size and appearance with the perithecia of the latter fungus, but open on the upper leaf surface. The conidia are hyaline, 27–36×1 μ, curved, one-septate in the middle, and contain several guttules.

Hypocreaceae

Hypocrea citrina (Pers. ex Fr.) Fries, Summa Veget. Scand. p. 185. 1846.

On old *Fomes* sp., *1915*, H. E. Bigelow *5428*.

Hypomyces aurantius (Fr.) Tulasne, Sel. Fung. Carp. 3: 43. 1865.

On old *Fomes applanatus* (Pers. ex Wallr.) Gill., *2014*.

Nectria modesta von Hoehnel, Sitz. K. Akad. Wiss. Wien, Math.-nat. Kl. 116(1): 106. 1907.

On other pyrenomycetes on *Abies balsamea* (L.) Mill., *2251*.

Nectria pedicularis (Tracy & Earle) Petrak, Hedwigia, 68: 230. 1929 (Figs. 29, 30).

On *Aster* sp., *2253*; *Epilobium angustifolium* L., *2255*; *Hieracium pratense* Tausch., *1908*; *Silene acaulis* L., *2279*.

These collections agree well with Petrak's (1929) detailed description of the species. In the material on *Silene*, the spores become rough-walled at maturity. In other respects that collection does not differ significantly from the other three. Tracy and Earle (1901) originally described the fungus from the mountains of Colorado and it has been reported elsewhere in the mountains of western North America. Petrak's collections were from Siberia.

Trichosphaeriaceae

Eriosphaeria vermicularia (Fr.) Sacc., Atti Soc. Ven. Trent. 4: 10. 1875 (Figs. 31, 32).

On fallen *Abies balsamea* (L.) Mill., *2251*.

Eriosphaeria vermicularia occurs on dead wood of various trees. It is well known in Europe, but not in North America. This collection agrees well with Mueller's (1953) description of the fungus.

The type specimen of *Venturia lanea* Dearness (1926) on bark of *Chamaecyparis nootkatensis* (Lamb.) Spach. from Washington, and a second specimen in the Dearness herbarium, on bark of *Picea engelmannii* (Parry) Engelm. from Idaho, are both identical with my collection from Quebec, and with Mueller's description. To the synonymy of *Eriosphaeria vermicularia* should be added:

Venturia lanea Dearness, Mycologia, 18: 246. 1926.
Cacosphaeria nectrioides Dearness ined. (in herbarium).

Niesslia pusilla (Fr.) Schroeter, Die Pilze Schlesiens, 2: 294. 1894.
On *Anaphalis margaritacea* (L.) B. & H., *2249*.

DIAPORTHACEAE

Gnomonia campylostyla Auerswald, Mycol. Europ. Heft V/VI: 25. 1869.
On *Betula* sp., *1900*, *1901*, *2039*.

Gnomonia intermedia Rehm, Ann. Mycol. 6: 489. 1908 (Figs. 33, 34).
On *Betula glandulosa* Michx., *1974*; *Betula* sp., *1900*, *1901*, *2039*, *2042*.

This fungus has the appearance of *G. campylostyla*, but the sizes throughout are much smaller. These collections of *G. intermedia* are identical with Rehm's (1908) description of the species. Apparently *G. intermedia* is rare, for I have found very few records of its occurrence, and these only from Germany.

Linocarpon umbelliferarum sp. nov. (Figs. 35–38).

Peritheciis 370–600 μ in latitudinem, 300 μ in altitudinem, globosis vel applanatis, immersis, supra in rostrum 77–308 μ altum, 80–100 μ crassum, erumpens attenuatis; peridiis brunneis. Ascis 67–97×6–7.5 μ, cylindriceis, ad apicem cum poro et sphaera refractaria praeditis, paraphysatis. Sporis 52–67× 1.5–2 μ, hyalinis vel subflavis, bacillaribus, 3-septis.

In caulo mortuis *Heraclei lanati* Michx. Specimen typicum legit M. E. Barr, n. *2198A*, loc. "Mont Albert, Gaspesian Park, Quebec", Augustus 19, 1957.

Perithecia 370–600 μ in diameter, $\pm$300 μ high, globose to applanate, immersed beneath epidermis, ostiole erumpent, 77–308 μ high, 80–100 μ in diameter, scattered to grouped, wall stout and dark brown, apex of beak composed of parallel hyphae, periphysate, few loose, branched, and contorted brown hyphae over surface. Asci 67–97×6–7.5 μ, cylindrical, apex rounded, base narrowed into a short stalk, wall thin and single, apex thickened and bearing a large refractive globule at tip of cytoplasm, a small refractive pore at apex, not turning blue in Melzer's solution, numerous, paraphyses few, delicate and thin-walled, guttulate. Spores 52–67×1.5–2 μ, hyaline with a faint yellowish tinge, filiform, ends rounded, width even throughout, straight to curved, three-septate, not constricted, wall thin and smooth, contents guttulate especially in end cells, in a parallel fascicle in the ascus.

On overwintered stalks of *Heracleum lanatum* Michx., *1951*, *2198A* (*type*).

This fungus presents a problem in generic position, with sphaeriaceous structure, no clypeus, and long filiform spores. It does not have the clypeus of *Linospora* nor the horizontal perithecium of *Plagiosphaera*. The sphaeriaceous structure immediately removes it from *Ophiobolus*. *Ceuthocarpon* has been described as a non-beaked, non-stromatic *Linospora*, but von Arx (1951) considered it synonymous with *Linospora*. The only possibility seems to be in the genus *Linocarpon*, according to Petrak's (1952) emended description of the genus. The fungus on *Heracleum* agrees in all points with the generic description, except that it does not possess the pseudoclypeus found in some species of *Linocarpon*. Munk's (1957) description of the ascus of *L. eucryptum* (Berk. and Br.) Petrak is identical with that observed here. However, *Linocarpon* as

presently recognized occurs only on monocotyledonous hosts. The new species, *Linocarpon umbelliferarum*, differs from all other species of the genus in that respect.

Melanconis alni Tulasne var. *marginalis* (Peck) Wehmeyer, A Revision of Melanconis . . ., Univ. of Mich. Press, 14: 27. 1941.
On *Alnus crispa* (Ait.) Pursh., *2178B*.

Phomatospora therophila (Desm.) Sacc., Syll. Fung. 1: 433. 1882.
On *Carex* sp., *2254*.

Von Arx and Mueller (1954) transferred this species to *Phyllachora* and noted a slight development of stromatic tissue around the perithecia. This collection, as well as others I have examined, shows only scattered brown hyphae in the host tissues and lacks a clypeus over the perithecium. I consider that the species should be retained in the genus *Phomatospora*.

Sydowiella fenestrans (Duby) Petrak, Ann. Mycol. 21: 30. 1923 (Fig. 39).
On *Epilobium angustifolium* L., *1911*, *1998*, *2255*.

Valsa ceratophora Tulasne, Sel. Fung. Carp. 2: 191. 1863.
On *Rubus strigosus* Michx., *1992*.

References

ARX, J. A. VON. 1951. Ueber die Gattung *Laestadia* und die Gnomoniaceen. Antonie van Leeuwenhoek. J. Microbiol. Serol. **17**, 259–272.

——— 1952. Studies on *Venturia* and related genera. Tijdschr. Plantenziekten, **58**, 260–266.

——— 1954. Revision einiger Gattungen der Ascomyceten. Acta Botan. Neerl. **3**(1), 83–93.

ARX, J. A. VON and MUELLER, E. 1954. Die Gattungen der amerosporen Pyrenomyceten. Beitr. Kryptogamenflora Schweiz, **11**(1), 1–434.

BARR, M. E. 1955. Species of sooty molds from western North America. Can. J. Botany, **33**, 497–514.

——— 1959. Northern pyrenomycetes I. Canadian eastern arctic. Contribs. inst. botan. univ. Montréal, **73**, 1–101.

DEARNESS, J. 1926. New and noteworthy fungi—IV. Mycologia, **18**, 236–255.

ELLIS, J. B. and EVERHART, B. M. 1892. The North American Pyrenomycetes. Newfield, N.J.

HOLM, L. 1957. Études taxonomiques sur les Pléosporacées. Symbolae Botan. Upsalienses, **14**(3), 1–188.

KARSTEN, P. A. 1883. Fragmenta mycologica IV. Hedwigia, **22**, 177–180.

LUTTRELL, E. S. 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies, **24**(3), 1–120.

MUELLER, E. 1950. Die schweizerischen Arten der Gattung *Leptosphaeria* und ihrer Verwandten. Sydowia, **4**, 185–319.

——— 1953. Ueber die Gattungen *Eriosphaeria* Sacc. und *Gaeumannia* Petr. Sydowia, **7**, 133–140.

——— 1954. Beiträge zur Kenntnis der Gattung *Gibbera* Fr. emend Petr. Sydowia, **8**, 60–73.

——— 1957. Systematische Bemerkungen ueber einige *Venturia*-Arten. Sydowia, **11**, 79–92.

MUELLER, E. and VON ARX, J. A. 1950. Einige Aspekte zur Systematik pseudosphaerialer Ascomyceten. Ber. schweiz. botan. Ges. **60**, 329–397.

MUNK, A. 1957. Danish Pyrenomycetes. Dansk Botan. Arkiv, **17**(1), 1–491.

PECK, C. H. 1873. Report of the Botanist. 25th Ann. Rept. N. Y. State Museum Nat. Hist. 57–123.

PETRAK, F. 1929. Mykologische Beiträge zur Flora von Sibirien. I. Hedwigia, **68**, 203–241.

——— 1941. Mykologische Notizen. XIV. Ann. Mycol. **39**, 251–349.

——— 1952. Ueber die Gattungen *Gaeumannomyces* v. Arx et Olivier, *Halophiobolus* Linder und *Linocarpon* Syd. Sydowia, **6**, 383–388.

REHM, H. 1908. Ascomycetes exs. Fasc. 42. Ann. Mycol. **6**, 485–491.

SCOGGAN, H. J. 1950. The flora of Bic and the Gaspe Peninsula, Quebec. Natl. Museum Can. Bull. **115**, 1–399.

SHEAR, C. L. 1948. Mycological notes. IX. Mycologia, **40**, 748–759.

TRACY, S. M. and EARLE, F. S. 1901. Fungi. *In* Plantae Bakerianae, **1**(1), 15–37.

WORONICHIN, N. N. 1926. *Aithalomyces*, eine neue Gattung der Capnodiales. Ann. Mycol. **24**, 145–149.

NOTE: Figs. 1–39 follow.

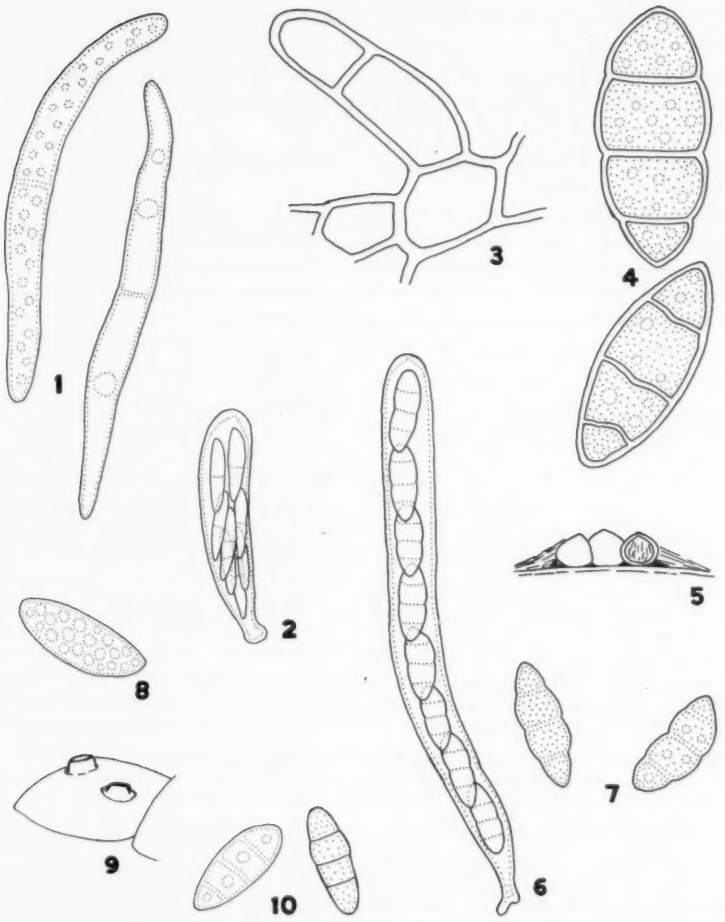
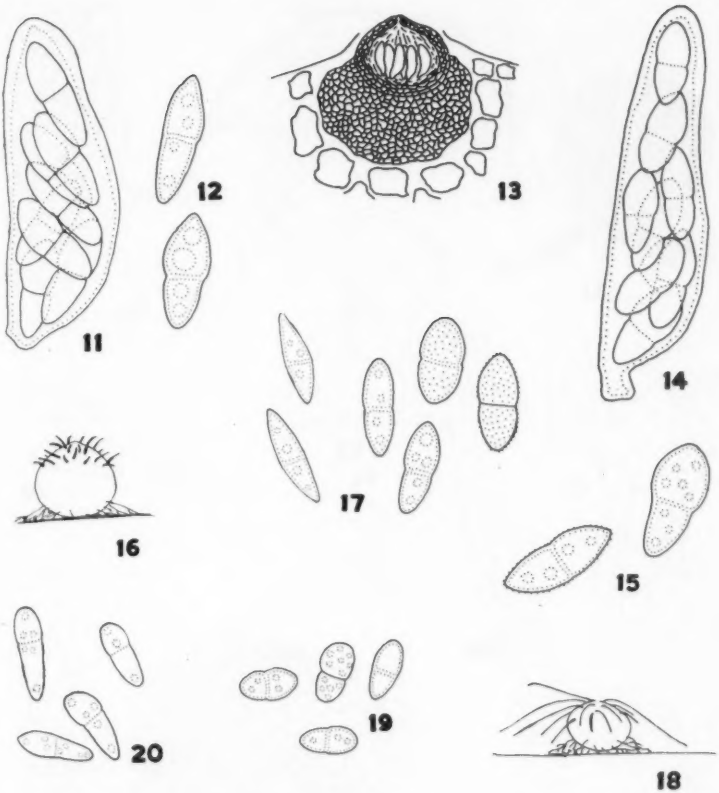
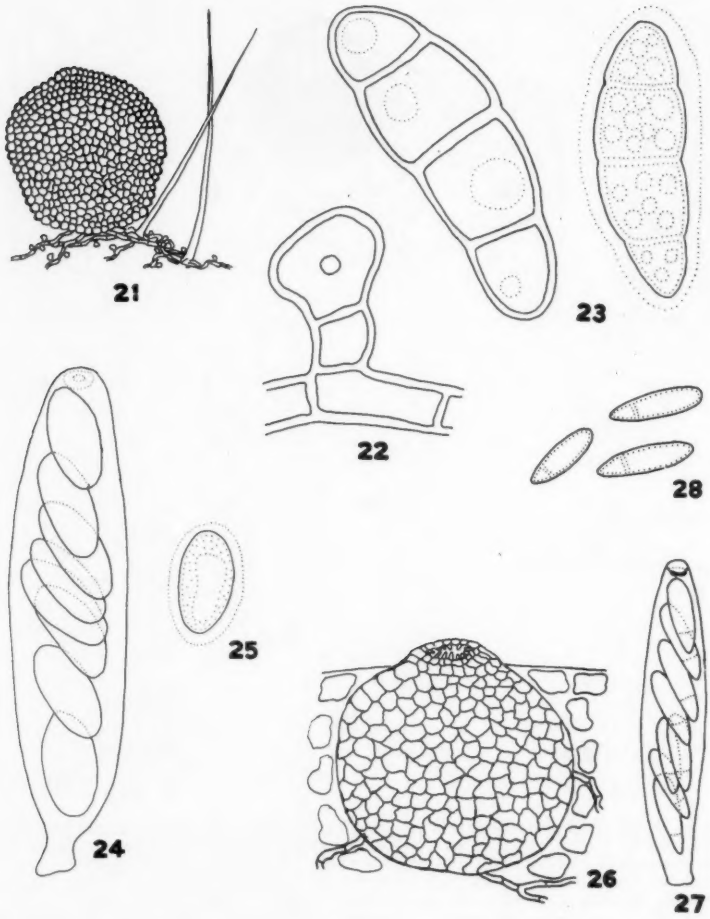


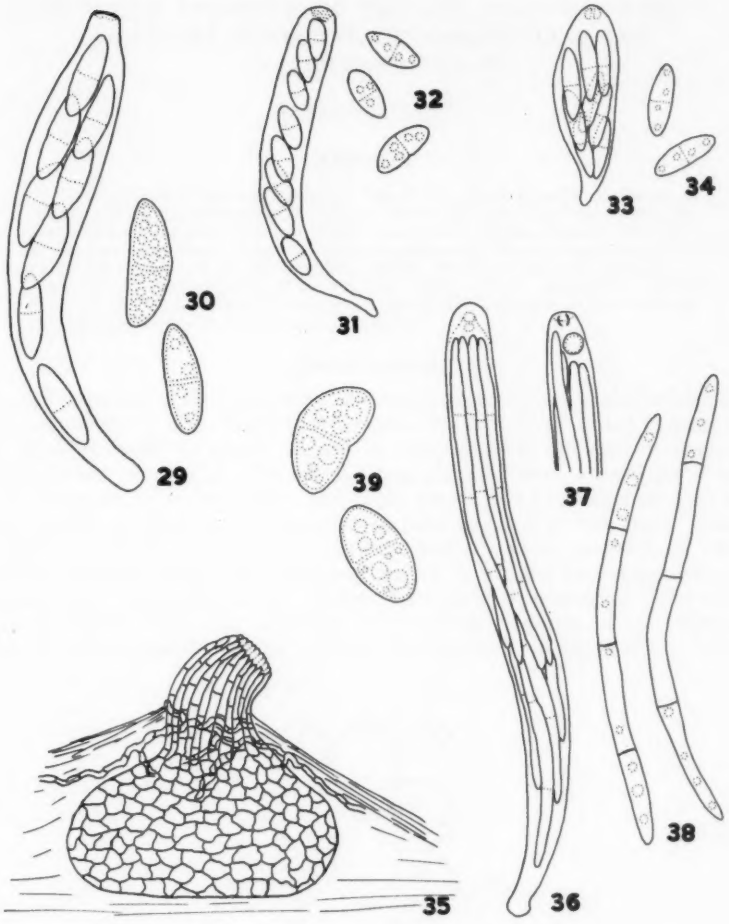
FIG. 1. *Wettsteinina niesslii* Mueller, ascospores (×1000).
FIG. 2. *Mycosphaerella colorata* (Pk.) House, ascus (×1000).
FIGS. 3, 4. *Limacinia arctica* (Wor.) Barr. Fig. 3 hyphal appendage, Fig. 4 ascospores (×1000).
FIGS. 5–7. *Gibberidea kalmiae* (Pk.) Barr. Fig. 5 habit of ascostromata (×20); Fig. 6 ascus, Fig. 7 ascospores (×1000).
FIG. 8. *Botryosphaeria abietina* (Prill. & Delacr.) von Arx & Mueller, ascospore (×1000).
FIGS. 9, 10. *Lophiostoma thujae* Ell. & Ev. Fig. 9 habit of compressed ascostromata on leaf (×20); Fig. 10 ascospores (×1000).



FIGS. 11, 12. *Gibbera cassandrae* (Pk.) Barr. Fig. 11 ascus, Fig. 12 ascospores (×1000).
FIGS. 13–15. *Gibbera grumiformis* (Karst.) Barr. Fig. 13 habit of glabrous ascostroma on hypostroma in leaf tissue (×100), Fig. 14 ascus, Fig. 15 ascospores (×1000).
FIGS. 16, 17. *Gibbera kalmiae* (Pk.) Barr. Fig. 16 habit of ascostroma (×100); Fig. 17 ascospores, showing variation in shape at different stages of maturity (×1000).
FIGS. 18, 19. *Venturia grossulariae* (Auersw. & Fleisch.) Sacc. Fig. 18 habit of ascostroma (×100); Fig. 19 ascospores (×1000).
FIG. 20. *Xenomeris raetica* (Mueller) Petrak, ascospores (×1000).



FIGS. 21–23. *Meliola nidulans* (Schw.) Cke. Fig. 21 habit of perithecium on setose subiculum (×100); Fig. 22 hyphopodium, Fig. 23 ascospores (×1000).
FIGS. 24, 25. *Physalospora vitis-idaeae* Rehm. Fig. 24 ascus, Fig. 25 ascospore (×1000).
FIGS. 26–28. *Pseudomassaria foliicola* Barr. Fig. 26 perithecium (×500); Fig. 27 ascus (the apical pore stained blue in Melzer's solution), Fig. 28 ascospores (×1000).



FIGS. 29, 30. *Nectria pedicularis* (Tracy & Earle) Petrak. Fig. 29 ascus, Fig. 30 ascospores (×1000).

FIGS. 31, 32. *Eriosphaeria vermicularia* (Fr.) Sacc. Fig. 31 ascus, Fig. 32 ascospores (×1000).

FIGS. 33, 34. *Gnomonia intermedia* Rehm. Fig. 33 ascus, Fig. 34 ascospores (×1000).

FIGS. 35–38. *Linocarpon umbelliferarum* Barr. Fig. 35 perithecium (×100); Fig. 36 ascus, Fig. 37 ascus tip in Melzer's solution showing refractive pore and large globule, Fig. 38 ascospores (×1000).

FIG. 39. *Sydowiella fenestrans* (Duby) Petrak, ascospores (×1000).

FURTHER EVIDENCE OF THE RELATION BETWEEN 2-DESVINYL-2-FORMYL-CHLOROPHYLL-*a* AND CHLOROPHYLL-*d*[1]

A. STANLEY HOLT

Abstract

2-Desvinyl-2-formyl-chlorophyll-*a* (made by permanganate oxidation of chlorophyll-*a*) and chlorophyll-*d* (isolated from *Gigartina papillata*) were compared. They possessed identical visible absorption spectra, formed reduction derivatives with identical spectra, and chromatographed as a single zone on a sugar column. These results provide further evidence that chlorophyll-*d* is 2-desvinyl-2-formyl-chlorophyll-*a*. Certain thalli of *G. papillata* yielded chlorophyll-*d* while others yielded none. All thalli of *Rhodomela larix* yielded chlorophyll-*d* but in extremely small amounts.

Introduction

It has been shown that 2-desvinyl-2-formyl-chlorophyll-*a* might be identical with chlorophyll-*d* (4). Attempts to confirm this conclusion failed because the red algae available for study yielded no chlorophyll-*d*. Subsequent inquiries revealed that samples of chlorophyll-*d* and pheophytin-*d* isolated previously (5, 6) were no longer available, and later, that other investigators also had been unable to confirm the presence of chlorophyll-*d* in red algae. Consequently, it was of interest to obtain samples of the same species from which chlorophyll-*d* had been previously isolated (5, 6, 8), and to obtain sufficient pigment for comparison with 2-desvinyl-2-formyl-chlorophyll-*a*. The latter pigment results from the oxidation of the 2-vinyl group of chlorophyll-*a* (Fig. 1) by potassium permanganate. The reaction yields first the 2-(1,2-

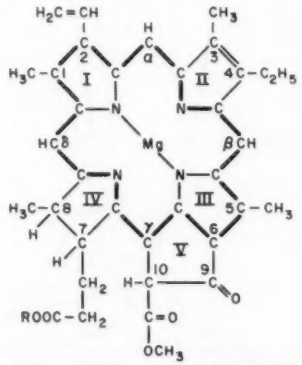


FIG. 1. Structure of chlorophyll-*a*.

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dihydroxy-ethyl) derivative, which is then oxidized to the 2-formyl derivative. This communication confirms the presence of chlorophyll-*d* in extracts of certain red algae and provides further support for the conclusion that chlorophyll-*d* is 2-desvinyl-2-formyl-chlorophyll-*a*.

Methods and Results

Samples of *Gigartina papillata* (C. A. Agardh) J. Agardh and *Rhodomela larix* (Turner) C. A. Agardh were collected at Moss Beach, San Mateo County, California, in Sept., 1959, from whole plants attached to rocks partially submerged at low tide. The material was stored 1–2 days in fresh sea-water at about 5° C. Thalli were blanched in boiling water for 1 minute (5) and were extracted with methanol at room temperature for 1–2 minutes. The light yellow-green solution was then examined with a Beckman recording spectrophotometer. The presence of a shoulder or peak at about 700 mμ on the long-wave-length side of the absorption maximum of chlorophyll-*a* indicated the occurrence of chlorophyll-*d* in the extract (5).

All extracts of randomly selected thalli of *R. larix* contained chlorophyll-*d*; the yields were low relative to the total amount of chlorophyll-*a* that could eventually be extracted. However, when thalli of *G. papillata* were extracted,

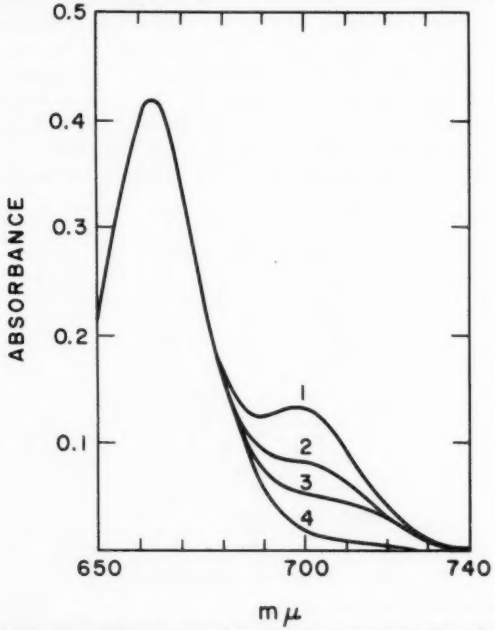

FIG. 2. The "red" absorption spectra of methanolic extracts of *Gigartina papillata*. The alga was extracted for 1 minute with four successive 10-ml volumes of methanol. Curve 1—first extraction; curve 2—second extraction; etc. The curves were adjusted 0.42 absorbance units at the "red" maximum of chlorophyll-*a*.

some yielded relatively large amounts while others yielded none. Figures 2 and 3 present examples of extractions of *G. papillata*. Extracts of both the lamina and the stipe contained chlorophyll-*d*.

To obtain chlorophyll-*d* in sufficient amount for comparison with 2-desvinyl-2-formyl-chlorophyll-*a*, approximately 2–3 kg wet weight of *G. papillata* was collected. Two-hundred-gram lots were blanched and extracted twice with 500 ml of methanol. The methanolic extracts were then added successively to about 1–2 liters of petroleum ether (b.p. < 50° C) in a separatory funnel. Unexpectedly, the lower yellow-colored methanolic phase was found to contain all the chlorophyll-*d*; none was detectable spectrophotometrically in the petroleum ether layer. Successive washes of the methanolic phase with fresh petroleum ether removed considerable chlorophyll-*a*, thereby increasing the relative concentration of chlorophyll-*d*. The total pigment in the methanolic

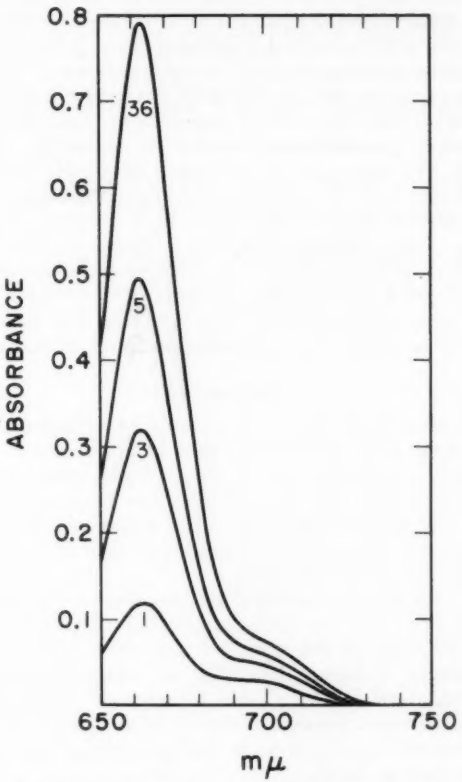

FIG. 3. The "red" absorption spectrum of a methanolic extract of *Gigartina papillata*. The alga was extracted with 100 ml of methanol. The absorbance was measured at the indicated number of minutes following the addition of methanol.

extract was then transferred into ether and evaporated to dryness. The absorbance at 690 mμ of the ether solution was about 1/10th that at 660 mμ.

The pigments were dissolved in petroleum ether and chromatographed on a powdered sugar column. At first chlorophyll-*a* and some yellow pigments moved down the sugar column as rapidly as the solvent front. After continued addition of petroleum ether to the column the pigments finally adhered to the sugar. Successive additions of 20, 40, 60, 80% (v/v) benzene in petroleum ether, followed finally by benzene alone, removed considerable yellow pigment. The column was extruded and cut into several segments. Chlorophyll-*d* was found in a zone extending from the top of the column to the top of the zone containing chlorophyll-*a*. Besides some yellow pigment this zone also contained a small amount of chlorophyll-*b*. The pigments were extracted with acetone and rechromatographed. By chromatographing twice more, using 0.1% (v/v) isopropanol in benzene, yellow pigments and traces of chlorophylls *a* and *b* were separated from chlorophyll-*d*. A fourth column for which 0.1% isopropanol in petroleum ether was used completed the purification.

The visible absorption spectra of ether solutions of chlorophyll-*d* and pheophytin-*d* were compared with those of 2-desvinyl-2-formyl-chlorophyll-*a* and its magnesium-free derivative, respectively, and were identical. The Molisch phase test intermediates in pyridine containing methanolic KOH (2, 9) were red. The spectrum of 2-desvinyl-2-hydroxymethyl-chlorophyll-*a* obtained by reduction of the synthetic sample with sodium borohydride (3) was identical with that of the reduction product obtained from chlorophyll-*d*. During storage, both chlorophyll-*d* and 2-desvinyl-2-formyl-chlorophyll-*a* changed partially into their "prime" derivatives (7). These were removed by column chromatography and equal amounts of the purified pigments were combined, and cochromatographed. Only one zone was detectable when the column was examined using 365 mμ radiation to excite fluorescence.

Discussion

The above results show that with respect to visible absorption spectra, reduction products, and chromatographic behavior on a sucrose column, chlorophyll-*d* and 2-desvinyl-2-formyl-chlorophyll-*a* possess identical properties. However, until sufficient chlorophyll-*d* has been isolated to permit the degradation to, and the identification of the imides of, all four pyrrole nuclei (1) and the characterization of the esterifying alcohol, it cannot be established that the two compounds are identical.

Two pertinent questions are raised by this study. (1) Is chlorophyll-*d* an artifact of extraction? (2) Why do certain thalli of *Gigartina papillata* yield relatively large amounts of chlorophyll-*d* and others yield none? With regard to the first question, it seems likely that the blanching process inactivated any enzymic oxidation of the C_2-vinyl group of chlorophyll-*a*, and that chlorophyll-*d* is, therefore, present in vivo. The answer to the second question will probably require a study of both the morphology and the physiological condition of the various thalli.

In any future study of this problem it will be of interest to determine whether any 2-desvinyl-2-(1,2-dihydroxy-ethyl)-chlorophyll-*a* is present in

the extracts. This compound has an absorption spectrum almost identical with that of meso-chlorophyll-*a*. As mentioned in the Introduction, it is the first product of the stepwise oxidation of chlorophyll-*a* to 2-desvinyl-2-formyl-chlorophyll-*a*.

Acknowledgments

The experiments described above were carried out at the Carnegie Institution of Washington, Division of Plant Biology. The author is most grateful to the Director, Dr. C. S. French, and to Dr. J. H. C. Smith for their encouragement and help, and to Dr. G. F. Papenfuss of the University of California, Berkeley, for identifying the algal specimens. To Mr. Guy C. McLeod go many thanks for assistance in the collecting of the algae.

References

1. FISCHER, H. and ORTH, H. 1934. Chemie des pyrroles. Vol. I. Leipzig. p. 396.
2. HOLT, A. S. 1957. The phase test intermediate and the allomerization of chlorophyll-*a*. Can. J. Biochem. and Physiol. **36**, 439–456.
3. HOLT, A. S. 1959. Reduction of chlorophyllides, chlorophylls and chlorophyll derivatives by sodium borohydride. Plant Physiol. **34**, 310–314.
4. HOLT, A. S. and MORLEY, H. V. 1959. A proposed structure for chlorophyll-*d*. Can. J. Chem. **37**, 507–514.
5. MANNING, W. M. and STRAIN, H. H. 1943. Chlorophyll-*d*, a green pigment of red algae. J. Biol. Chem. **151**, 1–19.
6. SMITH, J. H. C. and BENITEZ, A. 1955. Modern methods of plant analysis. Vol. IV. Springer-Verlag, Berlin. pp. 142–146.
7. STRAIN, H. H. and MANNING, W. M. 1942. Isomerization of chlorophylls-*a* and -*b*. J. Biol. Chem. **146**, 275–276.
8. STRAIN, H. H. 1958. Chloroplast pigments and chromatographic analysis. 32nd Annual Priestley Lecture, Pennsylvania State Univ., Univ. Park, Pa.
9. WELLER, A. 1958. The visible absorption spectra of the phase test intermediates of chlorophyll-*a* and -*b*. J. Am. Chem. Soc. **76**, 5819.

CHLOROPHYLL DERIVATIVES, SULPHUR, AND CARBON IN SEDIMENT CORES FROM TWO ENGLISH LAKES[1]

EVILLE GORHAM

Abstract

Chlorophyll derivatives, sulphur, and carbon exhibit similar trends in each of two sediment cores, one from the fertile Esthwaite Water and the other from the unproductive Ennerdale Water. In Esthwaite Water maximum levels of all three constituents were reached early in the course of lake development, while in Ennerdale Water maxima were attained much later, and, in the case of chlorophyll derivatives and sulphur, were much lower than in Esthwaite. Relatively high optical densities of acetone extracts between 400 and 500 mμ suggest the presence of carotenoids in surface muds from Esthwaite Water. Much lower optical densities were recorded for this wave-length range in extracts of deeper sediments from Esthwaite and all sediments from Ennerdale. Low ratios of optical density at 410 mμ to density at 350 mμ are characteristic of surface sediments from infertile lakes such as Ennerdale Water, and of woodland soils. However, higher ratios near to those observed throughout the Esthwaite core were found in the deeper Ennerdale sediments, with the exception of the deepest sample.

Ratios of chlorophyll derivatives to carbon and of sulphur to carbon were higher in the more fertile Esthwaite Water, although relatively low ratios during the earliest stages of development indicate that this lake may have been less fertile then. In Ennerdale Water the ratio of chlorophyll derivatives to carbon exhibited a much slower rise to maximum values than in Esthwaite, and these maxima were much the same as the early Esthwaite minima. The ratio of sulphur to carbon was also consistently lower in Ennerdale Water.

Introduction

Chlorophyll derivatives, sulphur, and carbon show marked intercorrelations in surface muds from five lakes in the English Lake District, and rise in concentration with increasing lake fertility (2). The present study examines these constituents in sediment cores from two of the above lakes, one shallow and fertile (Esthwaite Water) and the other deep and unproductive (Ennerdale Water). Some physical and chemical properties of these lakes are given in an earlier paper (2). In Esthwaite Water the bottom waters become anaerobic in summer, while in Ennerdale Water oxygen concentrations remain high (5).

Methods

Sediment cores down to the glacial deposits were taken from the deepest parts of the lakes with the Mackereth core sampler (3), and stored in a cold room. Fresh samples taken at irregular intervals down the core were analyzed for chlorophyll derivatives within a few days. Dried samples were stored for carbon and sulphur measurements. The analytical methods have been described (2). Relative concentrations of chlorophyll derivatives were estimated from the light absorption peak of 90% acetone extracts at about 667 mμ, and are reported in arbitrary units. One unit is equivalent to an optical density of

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Contribution from the Department of Botany, University of Toronto, Toronto, Ontario.

0.1 in a 1-cm cell when dissolved in 100 ml of solvent (cf. 8), after subtracting an approximate correction obtained by drawing a freehand baseline to the absorption curve between 550 and 750 mμ (cf. 6).

Results

Analytical results for the lake muds are presented in Fig. 1, on a dry weight basis. The first point to remark is the similarity of carbon concentrations in the upper muds of both lakes. Other cores from Esthwaite Water have shown up to twice the carbon concentrations of the present core, and Esthwaite surface muds are usually much richer in carbon than those of Ennerdale. Unfortunately it was not possible to analyze other cores before the author's departure from England, but the similarity of carbon values in these two cores accentuates the differences in their contents of sulphur and chlorophyll derivatives.

While carbon, sulphur, and chlorophyll derivatives follow similar trends, the two lakes exhibit marked dissimilarities in profile distribution of these three constituents. In Esthwaite Water all show a rapid rise to maximum levels rather deep in the mud column, especially in the case of carbon. The maximum for carbon is 9.5% dry weight at 340 and 380 cm depth, for sulphur 0.59% at 340 cm, and for chlorophyll derivatives 114 units per 100 g dry weight at 310 cm. In subsequent deposits a slight decline is evident in concentrations of all three constituents. In contrast to Esthwaite, maximum concentrations

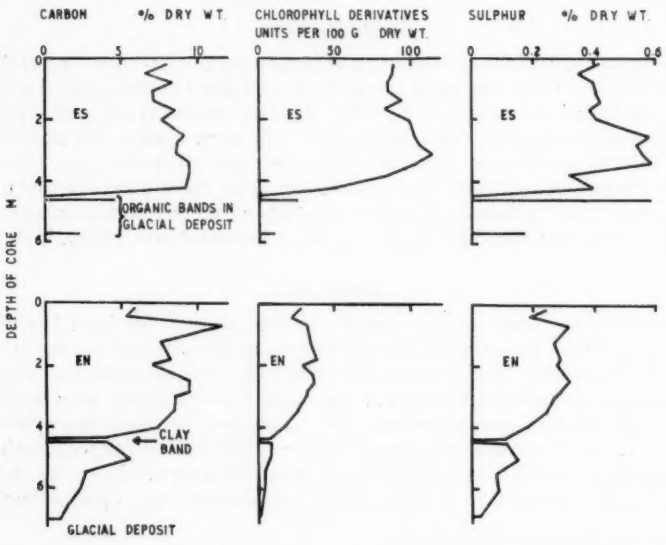

FIG. 1. The distribution of carbon, chlorophyll derivatives, and sulphur in cores from Esthwaite Water (ES) and Ennerdale Water (EN).

are reached much more slowly in Ennerdale and occur in the upper half of the mud column. In this case the maximum for carbon is 11.6% dry weight at only 70 cm depth, for sulphur it is 0.32% at 250 cm, and for chlorophyll derivatives 38 units per 100 g dry weight at 178 cm.

While carbon concentrations are generally similar in the upper parts of the two cores, that from the more fertile Esthwaite Water is much richer in both chlorophyll derivatives and sulphur. High levels of both these constituents, and high ratios to carbon, are associated with strongly reducing conditions (2), which are more likely to occur in the surface sediments and bottom waters of productive lakes such as Esthwaite Water than in those of infertile lakes like Ennerdale Water (5).

Examination of the absorption spectra of 90% acetone extracts, from which amounts of chlorophyll derivatives were estimated, presents interesting problems. In Fig. 2 are shown spectra for a surface sample (0–5 cm) from Esthwaite Water, a core sample from 570 cm in the same lake, a surface sample (0–5 cm) from Ennerdale Water, and another core sample from 550 cm in the latter lake. All have been adjusted to give equal optical densities for the red peak between 665 and 670 mμ. The first spectrum is similar to others from samples taken at 0–1, 6–7, and 9–10 cm in Esthwaite Water, while the second is much the same as spectra from Esthwaite core samples at 250 and 380 cm. The surface Ennerdale sample provides a spectrum only slightly different from those at 0–1 and 10–11 cm, while the spectrum of the deep sample closely resembles that of another sample at 300 cm, and yet another at only 16 cm depth. The points of resemblance between these spectra and that of pheophytin *a* have been described in the earlier study of surface muds (2, cf. also 6).

It is apparent that the surface Esthwaite spectrum shows much greater optical density in the region of 400–500 mμ than does the deeper spectrum.

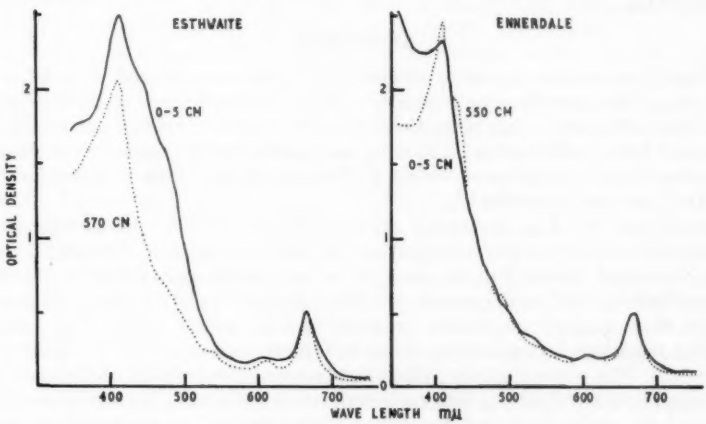

FIG. 2. Absorption spectra of 90% acetone extracts of muds from different depths in Esthwaite Water and Ennerdale Water.

If the values for the deep core are subtracted from those of the surface mud (cf. 8), the difference curve exhibits a distinct maximum optical density at 450 mμ, and a shoulder at 470–480 mμ, suggesting (cf. 7) that carotenoid pigments may be present in the surface deposit to a much greater degree than below. If so, it is impossible at present to state whether or not they were initially high in the sample at 570 cm depth, and destroyed during diagenesis; but the problem deserves further investigation. Neither surface nor deep Ennerdale samples show such high optical densities at 450–480 mμ as does the Esthwaite surface sample. Surface muds in Ennerdale are much better aerated than those of Esthwaite (5), so that carotenoids might be more liable to oxidative destruction in the former lake.

Work on surface muds (2) suggested that the ratio of optical density at 410 mμ to that at 350 mμ rises with increasing lake fertility. However, while surface samples from 0–1, 5–6, and 0–5 cm depth in Ennerdale yielded low ratios of 1.1, 0.8, and 0.9 respectively, the 18 samples from 16 cm downward in the Ennerdale core gave ratios ranging from 1.2 to 1.5, except for the deepest sample, which yielded a ratio of only 0.8. Esthwaite core ratios of 1.4, 1.4, 1.6, 1.6, and 1.4 were recorded from 140-, 250-, 380-, 460-, and 570-cm depths respectively. The subsurface Ennerdale core ratios are therefore only slightly lower than those of Esthwaite, and they also fail to show any correlation with either carbon content or the amounts of chlorophyll derivatives in the mud. Possibly the high optical densities shown by extracts of Ennerdale surface mud at 350 mμ are due to substances produced by oxidation in the relatively aerobic uppermost deposits in this lake; and these substances might subsequently be reduced when further sedimentation has led to development of anaerobic conditions in the former surface layer. Spectra from extracts of aerobic woodland soils resemble those from surface Ennerdale muds in exhibiting high optical densities at 350 mμ, but are even more extreme in this respect (1).

Discussion

Calculation of the amounts of chlorophyll derivatives and of sulphur per unit of carbon reveals some points of interest, as shown in Fig. 3. Not only is the Esthwaite core richer than the Ennerdale core in absolute terms, but the ratios of both constituents to carbon (and therefore to total organic matter) are also higher in the more fertile Esthwaite Water. This is in accord with results from surface muds (2).

For Ennerdale, Fig. 3 shows a gradual increase in the ratio of chlorophyll derivatives to carbon from the bottom of the core upward, to levels varying between about 3 and 5 units per g of carbon in the upper half of the core. If results from surface deposits in the five lakes previously studied (2) are any guide, this suggests a gradual increase in lake productivity. The ratio of sulphur to carbon is more constant, with sulphur ranging between 2.4 and 4.1% of carbon. The percentage is rather high toward the bottom of the core, and perhaps in these relatively inorganic sediments there may be a greater contribution from pulverized rock flour than in the upper and more organic muds. The minimum percentage of sulphur is found at 400 cm depth, above which levels tend to increase toward the surface. There is, however, a low value

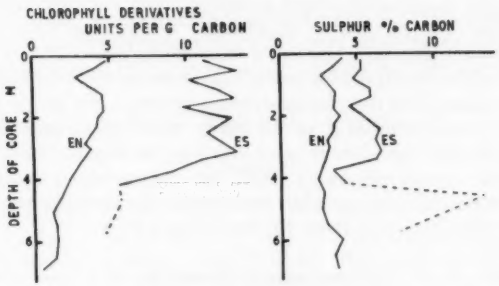

FIG. 3. Chlorophyll derivatives and sulphur calculated per unit of carbon in cores from Esthwaite Water (ES) and Ennerdale Water (EN). Interrupted lines join thin sediment bands to continuous mud column.

(2.7%) at 70 cm, where organic carbon is maximal; and at this depth the content of chlorophyll derivatives in the organic matter also shows a marked drop (to 2.8 units per g carbon).

In Esthwaite Water the content of chlorophyll derivatives per unit organic matter is lower in the two organic bands at the bottom of the core, and in the bottom sample from the continuous mud column, than in the muds above. However, the levels toward the bottom of the core are about the same as the maximum levels attained in Ennerdale Water, ranging from 4.6 to 5.6 units per g of carbon. This suggests that Esthwaite at its least productive may have been as fertile as Ennerdale at its best. A sharp rise occurs in subsequent Esthwaite deposits, to levels of about 10–13 units of chlorophyll derivatives per g of carbon throughout the top 3 m of the core. These values are similar to those obtained from six surface samples of distinctly higher organic content, whose carbon concentrations ranged from 11 to 13% dry weight.

The lower concentrations of chlorophyll derivatives in the organic matter of the earliest deposits indicate that Esthwaite Water may have been less productive in its early than in its later stages of development. Even where carbon concentration reached 9.3% dry weight, at 420 cm, there were only 5.5 units of chlorophyll derivatives per g of carbon. According to Mackereth (4), the exceptionally high levels of manganese toward the bottom of Esthwaite mud cores suggest that these early deposits did not become reduced right to the surface during summer, as they do at the present day; and this supports the view that the lake was less productive then. Further evidence comes from the deepest horizons of the continuous mud column, where sulphur also is low, being 4.3% of carbon at 420 cm depth, and 3.4% at 380 cm, in contrast with levels above 6% in the immediately succeeding deposits. A slight decline in sulphur concentrations occurred as sedimentation proceeded, to levels of about 5–6% of carbon in the uppermost muds.

It may be noted that the thin organic bands in the glacial deposits of Esthwaite Water are relatively rich in sulphur, especially the upper one. While much of the sulphur in the mud column above is no doubt of organic origin, presumably a good deal of the sulphur in the thin mud bands has

migrated in along a negative redox gradient from the pulverized rock flour above and below. In the case of Esthwaite Water this rock flour must be largely derived from the sedimentary rocks of the drainage basin; Ennerdale Water, on the other hand, drains mainly igneous strata. In this connection, too, the upper material of the glacial deposit between the two organic bands is probably much less weathered than the lower, since the former effervesces with hydrochloric acid and therefore retains much of its original calcium carbonate (Mackereth, oral communication). Such an unweathered sedimentary rock flour might well contain appreciable amounts of sulphur, as suggested by the high concentrations recorded from the mud band in it.

Concluding Remarks

From the foregoing results, taken together with those from surface deposits (2), it would seem reasonable to conclude that high ratios of chlorophyll derivatives to carbon may indicate productive phases in the history of a lake, while low ratios reflect periods of relative infertility. If so, it is noteworthy that the more fertile lake, Esthwaite Water, developed rapidly toward a more or less steady level of high productivity, while the relative sterility of Ennerdale Water entailed a much slower development to its present low level of productivity.

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References

1. GORHAM, E. 1959. Chlorophyll derivatives in woodland soils. Soil Sci. **87**, 258–261.
2. GORHAM, E. 1960. Chlorophyll derivatives in surface muds from the English Lakes. Limnology & Oceanog. **5**, 29–33.
3. MACKERETH, F. J. H. 1958. A portable core sampler for lake deposits. Limnology & Oceanog. **3**, 181–191.
4. MACKERETH, F. J. H. 1959. In the section on chemistry. 27th Ann. Rept. Freshwater Biol. Assoc. Brit. Empire.
5. MORTIMER, C. H. 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol. **30**, 147–201.
6. ORR, W. L. and GRADY, J. R. 1957. Determination of chlorophyll derivatives in marine sediments. Deep-Sea Research, **4**, 263–271.
7. RICHARDS, F. A. 1952. The estimation and characterization of plankton populations by pigment analyses. J. Marine Research, **11**, 147–155.
8. VALLENTYNE, J. R. 1955. Sedimentary chlorophyll determination as a paleobotanical method. Can. J. Botany, **33**, 304–313.

THE EFFECT OF 3-AMINO-1,2,4-TRIAZOLE ON THE UPTAKE, RETENTION, DISTRIBUTION, AND UTILIZATION OF LABELLED PHOSPHORUS BY YOUNG BARLEY PLANTS[1]

D. J. WORT[2] AND B. C. LOUGHMAN[3]

Abstract

Seedlings of barley were grown to the 3-leaf stage in phosphate-free nutrient solution and AT was added at such times that the roots were in contact with 100 p.p.m. of the compound for 96, 48, 24, 4, 1, or 0 hours immediately prior to transfer to labelled phosphate solution for 1 hour. The plants were then returned to phosphate-free nutrient solution and harvested 1, 2, 4, 24, 48, and 96 hours after the start of the contact with radioactive solution. In an experiment designed to determine the effect on utilization of phosphate the immersion in AT was of 48 hours' duration.

Exposure to AT diminished uptake of P^{32} but also decreased the subsequent loss to the environment. The activity in both acid-soluble and acid-insoluble fractions of the root was reduced by treatment, but exposure to AT for up to 48 hours increased the amount of P^{32} translocated to the shoot. The proportion of the absorbed phosphate found in the shoots of treated plants was higher than in the controls.

For 2 to 4 hours the incorporation of P^{32} into both acid-insoluble (nucleic acids, phospholipids, phosphoprotein) and acid-soluble (nucleotides, sugar phosphates, inorganic phosphate) fractions showed little deviation from normal, but by the end of 24 hours AT treatment caused a decrease in the acid-insoluble fraction of both roots and shoots. The pattern of esterification was changed but little.

Apparently the effect of AT on phosphate metabolism is not concerned with the reactions of oxidative phosphorylation or of glycolysis, but is confined to the processes involved in the incorporation of phosphate during the synthesis of nucleic acids.

The compound 3-amino-1,2,4-triazole (AT), widely used as a herbicide and defoliant, affects a number of plant metabolic processes. The characteristic chlorosis consequent to the application of AT to a variety of plants has been shown to result from a reduction or lack of chloroplast formation (9). Sund (12) suggested that a blocking of riboflavin synthesis may account for the chloroplast effects. His experimental results indicate that AT may also interfere with normal purine metabolism.

AT application has increased the respiratory rates of *Avena* sections and cotton leaf disks (7), bean and wheat leaves (13), corn plants (6), and timothy seedlings (10). A drastic decrease in rate of photosynthesis by leaves of wheat and bean treated with AT has also been described (13).

As shown by Pyfrom *et al.* (8), AT depresses catalase activity in barley and potato plants. Inhibition of algal phosphorylase by the chemical was demonstrated by Fredrick and Gentile (1). Polyphenol oxidase activity is lower in the chlorotic tissues of treated corn plants but cytochrome oxidase is not

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affected (6). The addition of 10^{-8} *M* AT to homogenates of Marquis spring wheat seedlings grown in the dark for 6 days resulted in an increased rate of oxidation of reduced cytochrome *c* (13).

Herbert and Linck (2) have investigated the action of AT on the level of phosphorus (P) in Canada thistle (*Cirsium arvense*). Application of AT to leaf disks of P-deficient plants did not stimulate the rate of respiration as it did when applied to disks of plants receiving adequate P. When P^{32} was applied to the foliage of P-deficient plants, the uptake was greatly reduced if the plants had been treated with AT.

An analysis of the various effects of AT application suggests that they may indeed stem, in part, from an altered P economy that extends from intake of P by the plant, through nucleotide and phosphate ester formation to the synthesis of protein. The present paper describes an attempt to discover the influence of AT, taken in through the roots of young barley plants on (1) the uptake of P from nutrient solution, (2) the ability of the plant to prevent the subsequent loss of the absorbed P to nutrient solution, (3) the distribution of the absorbed P between the roots and leaves of the plant, and (4) the incorporation of the P into the acid-insoluble form (nucleic acids, phosphoproteins, and phospholipids) and three major fractions of the acid-soluble compounds of this element—nucleotides, sugar phosphates, and inorganic phosphates.

Methods

Experiment 1: Absorption, Retention, and Distribution of P^{32} Between Root and Shoot

Twelve seedlings of barley (var. Proctor) were grown in each of 18 polystyrene tanks containing continuously aerated phosphate-free culture solution (11). Fifteen days after germination (early three-leaf stage) AT was added to the culture solution of groups of three tanks at such times that the roots of the various groups of plants were exposed to the AT for 96, 48, 24, 4, 1, or 0 hours prior to transfer of the plants to labelled phosphate solution. The concentration of AT in the tanks was 100 p.p.m.

At midday, the roots of the plants were quickly and thoroughly rinsed in demineralized water and all were simultaneously immersed in fresh culture solution to which had been added KH_2PO_4 and $KH_2P^{32}O_4$ to give a concentration of 10^{-5} *M* phosphate at pH 5.5 and an activity of 18 μc P^{32} per tank of 800 ml culture solution. After an exposure of 1 hour to the solution containing P^{32} all plants were returned, after the roots were rinsed in demineralized water, to fresh phosphate-free culture solution.

Three pairs of plants were removed from each of the groups which had received the various AT treatments 1, 2, 4, 24, 48, and 96 hours after the start of the contact with P^{32}. The plants were quickly divided into roots and shoots and placed in a 105° C oven to dry to constant weight. After drying, the roots and the shoots of the pairs of plants were weighed and wet ashed with a mixture of 2.2 ml 60% perchloric acid and 0.5 ml nitric acid (S.G. 1.42). The digest was made up to 10 ml with water and counting was done in a 20th Century Electronics M 6 liquid counter.

In effect, therefore, plants whose roots had been in contact with 100 p.p.m. AT for 96, 48, 24, 4, 1, or 0 hours were simultaneously placed in the P^{32} solution,

allowed to absorb phosphate for 1 hour, returned to P-free culture solution, and then 1, 2, 4, 24, 48, and 96 hours after the start of contact with P^{32} three groups of two plants from each of the six AT treatments were harvested for counting. The values included in the figures and table are the averages of these three replicates.

Experiment 2: The Distribution of P^{32} in Various Phosphorus Compounds

Barley seedlings were grown in phosphate-free nutrient solution as before. When the plants were 13 days old and at the three-leaf stage AT was added to the nutrient solution in half of the tanks to give a concentration of 100 p.p.m. Forty-eight hours later all the plants were transferred to nutrient solution containing 1×10^{-5} *M* phosphate, as in the first experiment, but with an activity of 80 μc P^{32} per 800 ml nutrient solution. At the end of 1 hour contact with the P^{32} the plants were transferred to fresh phosphate-free nutrient. Two groups of 12 plants were removed from the AT-treated and from the control lots 1, 2, 4, and 24 hours after the start of P^{32} absorption, for extraction of phosphorus compounds.

Extraction

The methods used for the extraction of compounds of phosphorus from the plant tissue were modifications of the procedures of Loughman and Martin (3). The two groups of 12 plants from the AT-treated and control groups were quickly divided into roots and shoots. These parts, cut into short pieces, were immersed in 12 ml of ice-cold 16% trichloracetic acid (TCA) in chilled mortars, causing an immediate cessation of metabolic activity. Grinding was continued for 5 minutes. The insoluble material was removed by centrifugation in chilled tubes for 7 minutes at 3750 r.p.m. The supernatant solution was decanted and the debris re-extracted with 5 ml of ice-cold 5% TCA and centrifuged as above. The supernatant solutions were combined and made up to 25 ml with water. A sample of this solution, containing nucleotides, sugar phosphates, and inorganic phosphate, was used for counting in an M6 liquid counter.

The TCA was removed from a 10-ml sample of the acid-soluble solutions by a 3-hour ether extraction in a continuous liquid–liquid extraction system, giving a final pH of 3.5. Any material precipitated during this extraction was removed by centrifugation. The low pH insured that coprecipitation of phosphorus compounds would be minimal.

The TCA-free solution was used in the paper chromatographic separation of the main groups of the acid-soluble components. The solid cell material, containing nucleic acids, phosphoproteins, and phospholipids, was transferred to Kjeldahl flasks and digested in perchloric–nitric acid (5.0 ml + 5.0 ml). After cooling, the digest was made up to 25 ml with water and counted in an M6 liquid counter.

Separation by Paper Chromatography

Apart from small modifications, the method of Loughman and Martin (3) was employed in the chromatographic separation of the acid-soluble phosphate groups. The solvent used was tert-butanol (80 ml)/water (20 ml)/picric acid (2.0 g). Two-milliliter samples were reduced in volume and spotted on acid-washed 3 MM Whatman paper. The chromatogram strips were passed through

an automatic recording scanner to measure and record peaks of activity, and autoradiographs were then prepared.

The autoradiographs and records were used in the division of the chromatogram strips into segments containing nucleotides, sugar phosphates, and inorganic phosphate. The segments were wet ashed in Kjeldahl flasks using perchloric acid and nitric acid (2.2 ml + 2.0 ml). The digests were made up to 10 ml and the counting carried out in an M6 liquid counter.

For further characterization of the nucleotides and sugar phosphates additional chromatograms were run and these fractions eluted from strip segments with water. The solutions were transferred to washed Whatman 3 MM filter paper and separated by the use of *n*-propanol (16 ml)/0.880 ammonia (30 ml)/water (10 ml) as solvent. One-hundred-microgram quantities of versene were applied to the spots on the paper to improve the running in the alkaline solvent. Autoradiographs and scanner records were made of these chromatograms.

The actual phosphorus content of the roots and shoots was not determined, but the relative activities of the parts were considered to indicate the relative intake of phosphorus by the roots and its distribution throughout the plant. Treatment with AT was found to have little effect on the distribution of phosphate already present in the plant at the time of treatment. Loss of labelled phosphate from the roots to the nutrient solution was followed by assay of the solution at the times of harvest.

In the text below the abbreviations 1-hr, 4-hr, 24-hr, and 96-hr are used to indicate those barley plants whose contact with the 100 p.p.m. AT solution was of the designated duration.

Results

The appearance of the barley plants at the conclusion of the experiment was: 96-hr, practically devoid of green color, little living tissue evident, no growth; 48-hr, distal two-thirds of the leaves brown, rest faded green, little growth; 24-hr, similar to 48-hr, but less fading of chlorophyll; 4-hr, lighter green than controls, leaves somewhat curved, chlorophyll breakdown in longitudinal streaks; 1-hr, distal one-third of leaves showing chlorophyll breakdown in streaks. Whereas the control plants increased their dry weight steadily during the course of the experiment, growth of the plants to AT for the longer periods was curtailed and completely prevented by the 96-hour contact with the chemical.

Intake of P^{32} and Subsequent Loss to the Nutrient Solution

The content of P^{32}, as activity per plant, expressed as millimicrocuries (mμc), is given in Fig. 1. In all cases except for the 1-hour contact with AT, exposure of the roots to the compound resulted in a diminution of phosphate uptake. The decrease in absorption became greater as the length of exposure to AT increased. At the conclusion of the 1-hour absorption period in the second experiment the total activity of control and treated plants was 920.4 and 841.8 mμc per plant respectively. In this case AT treatment reduced intake by 8.5% (Table I).

Outward movement of the P^{32} (which had been absorbed during the 1-hour

contact with the nutrient solution containing phosphate) from the roots into the final nutrient solution lacking phosphate occurred from all plants. It was most rapid during the 3-hour period immediately after removal from the P^{32} solution (Fig. 1). The actual amount of P^{32} lost to the solution was greatest in the control and 1-hour plants. The percentages, based on the activity in the plant at the end of 1-hour exposure to labelled phosphorus, lost to the minus-P nutrient solution by the 96th hour were:

control	24.1,
1-hour	33.7,
4-hour	7.0,
24-hour	4.6,
48-hour	13.1,
96-hour	30.7.

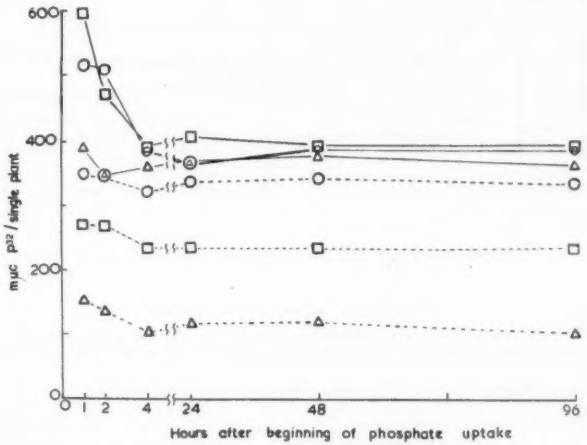

FIG. 1. The effect of AT on the subsequent uptake of phosphate by whole barley plants during a 1-hour absorption period in 10^{-5} M $KH_2P^{32}O_4$, and their content thereafter. Duration of treatment with AT prior to phosphate absorption:

△ - - - - - △ 96 hours
□ - - - - - □ 48 hours
○ - - - - - ○ 24 hours
△ ——— △ 4 hours
□ ——— □ 1 hour
○ ——— ○ Control

A steady state was reached in all cases 3 hours after removal of the plant from the P^{32} nutrient, as shown by the horizontal portion of the curves in Fig. 1. The activity in the treated plants at the beginning of the steady state, expressed as percentage of the activity in the control plants was:

1-hour	102.0,
4-hour	93.4,
24-hour	83.1,
48-hour	60.8,
96-hour	26.3.

The effect of AT to decrease the intake of P^{32} and to enhance the ability of the plant to retain the absorbed P^{32} is evident from these two sets of figures.

Distribution of P^{32} in Roots and Shoots

Roots

The highest activity in the roots of all plants was found at the end of the 1-hour exposure to P^{32} (Fig. 2). This decreased rapidly for the first 3 hours after the plants were removed from the phosphate solution. Almost all of the P^{32} which left the root during this period was translocated into the shoot.

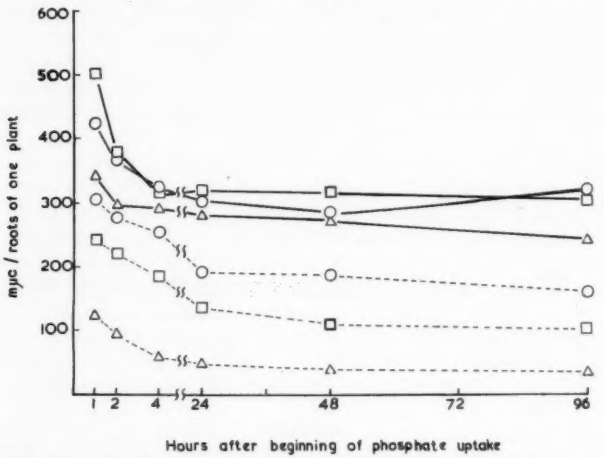

FIG. 2. The effect of AT on the subsequent uptake of phosphate by the roots of whole barley plants during a 1-hour absorption period in 10^{-5} *M* $KH_2P^{32}O_4$ and their content thereafter.

Duration of treatment with AT prior to phosphate absorption:

△ - - - - - △ 96 hours
□ - - - - - □ 48 hours
○ - - - - - ○ 24 hours
△ ——— △ 4 hours
□ ——— □ 1 hour
○ ——— ○ Control

Shoots

The shoots of control and 1-hour plants had the greatest P^{32} content at the end of the 1-hour exposure to phosphate (Fig. 3). In the case of the 2-, 4-, 24-, and 48-hour plants the activity at this time was below that of control, but the P^{32} continued to move out of the roots into the leaves of these treated plants during the duration of the experiment. Ninety-six hours after the first contact with P^{32} the activity in the shoots, as mμc per plant, and this activity expressed as percentage of the total activity in the plant were:

control	71.7 mμc	18.3%,
1-hour	93.0 "	23.5,
4-hour	121.5 "	30.7,
24-hour	173.6 "	52.0,
48-hour	131.6 "	56.2,
96-hour	66.6 "	65.7.

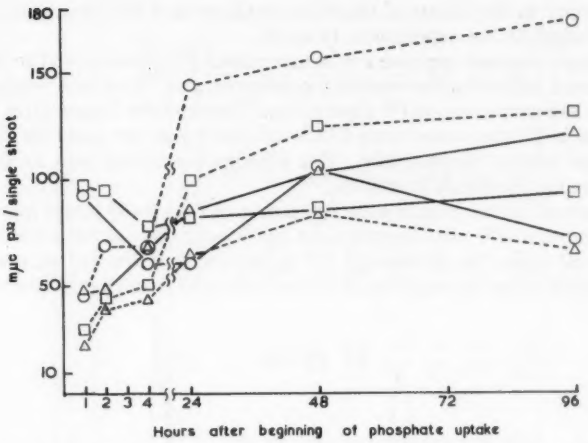

FIG. 3. The effect of AT on the transfer of phosphate to the shoots of barley plants during and after a 1-hour absorption period in 10^{-5} *M* $KH_2P^{32}O_4$.

Duration of treatment with AT prior to phosphate absorption:

△ - - - - - △ 96 hours
□ - - - - - □ 48 hours
○ - - - - - ○ 24 hours
△ ——— △ 4 hours
□ ——— □ 1 hour
○ ——— ○ Control

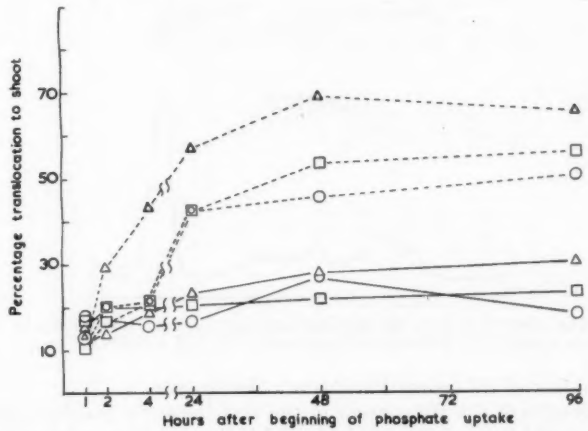

FIG. 4. The effect of AT on the percentage of the total absorbed phosphate translocated to the shoots of barley plants during and after a 1-hour absorption period in 10^{-5} *M* $KH_2P^{32}O_4$.

Duration of treatment with AT prior to phosphate absorption:

△ - - - - - △ 96 hours
□ - - - - - □ 48 hours
○ - - - - - ○ 24 hours
△ ——— △ 4 hours
□ ——— □ 1 hour
○ ——— ○ Control

The activity in the shoots of the 96-hour plants was less than that in control shoots throughout the experiment (Fig. 4).

There was a marked increase in the amount of P^{32} translocated to the shoots of AT-treated barley in the second experiment also. This was evident at the end of the 1-hour period of P^{32} absorption. Twenty-four hours after the start of exposure to P^{32} the values were 139.3 and 350.1 mμc per plant for the control and treated plants respectively. The enhanced activity was in both acid-insoluble and acid-soluble fractions.

Treatment of barley plants with 100 p.p.m. AT up to 48 hours increased the actual amount of P^{32} that moved into the shoots, compared with untreated plants. In all cases the percentage P^{32} in the shoot of treated plants, relative to the activity in the entire plant, was also increased over that of control plants.

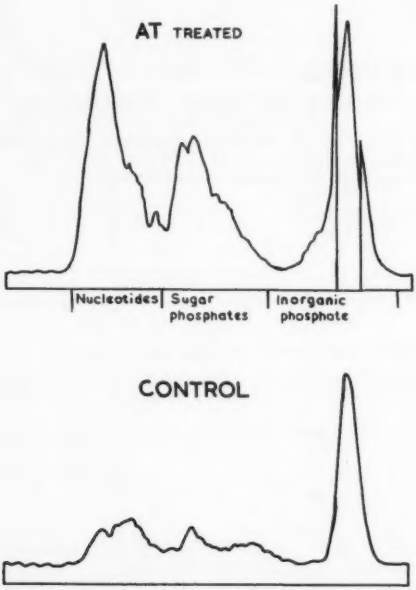

FIG. 5. The effect of AT on the distribution of acid-soluble phosphorus in shoots of barley plants 1 hour after immersion of the roots in 10^{-5} *M* $KH_2P^{32}O_4$.

The Distribution of P^{32} among Phosphorus Compounds

The expression of the P^{32} content of the acid-insoluble (nucleic acids, phospholipids, phosphoproteins) and acid-soluble (nucleotides, sugar phosphates, inorganic phosphates) fractions in the roots and in the stems as percentages of the total P^{32} content of these organs revealed that treatment resulted in no essential change in distribution of P^{32} between these two fractions for 2 hours in the roots and for 4 hours in the shoots (Table I). With time thereafter, though an increasing percentage of P^{32} was incorporated into the acid-insoluble

TABLE I

The effect of treatment with AT on the subsequent absorption, utilization, and esterification of phosphate. AT treatment 48 hours, followed by 1 hour in 1×10^{-5} *M* KH_2PO_4 and transfer to phosphate-free culture solution

Hours after initial contact with 1×10^{-5} *M* phosphate containing P^{32}			Total activity (mμc/organ)	% translocation to shoot	Acid-insoluble activity (mμc/organ)	Acid-soluble activity (mμc/organ)	Acid-insoluble as % total activity	Acid-soluble as % total activity	% acid-soluble activity in the form of:			% esterification (nucleotides + sugar phosphates)
									nucleotides	sugar phosphates	inorganic phosphates	
1	T*	Root	538.1		87.6	450.5	16.5	83.5	41.8	19.2	39.0	61.0
		Shoot	303.7	35.1	40.0	263.7	11.8	88.2	27.0	20.5	52.5	47.5
	C†	Root	866.8		310.6	556.2	35.4	64.6	43.9	24.3	31.8	68.2
		Shoot	53.6	5.7	6.6	47.0	12.2	87.8	30.2	19.8	50.0	50.0
2	T	Root	342.0		121.4	220.6	35.5	64.5	48.8	22.3	28.9	71.1
		Shoot	178.7	33.5	31.7	147.0	17.7	82.3	30.7	23.0	46.3	53.7
	C	Root	949.7		264.6	418.1	38.8	61.2	52.7	25.6	21.7	78.3
		Shoot	153.4	16.4	19.9	113.5	14.9	85.1	29.8	21.7	48.5	51.5
4	T	Root	322.8		111.2	211.6	34.2	65.8	47.3	25.5	27.2	72.8
		Shoot	261.3	45.1	56.1	206.2	21.3	78.7	31.1	24.2	44.7	55.3
	C	Root	609.3		304.6	304.7	50.2	49.8	58.9	19.7	21.9	78.1
		Shoot	149.7	18.0	26.1	123.6	17.4	82.6	28.9	20.3	50.8	49.2
24	T	Root	201.2		113.0	84.2	57.1	42.9	44.0	24.6	31.4	68.6
		Shoot	350.1	68.4	140.5	209.6	41.4	58.6	29.5	21.7	48.8	51.2
	C	Root	437.0		324.0	113.2	74.1	25.9	55.2	23.1	21.7	78.3
		Shoot	139.3	24.2	82.4	56.9	59.2	40.8	37.8	20.9	41.4	58.6

*T = treated with AT.
†C = control.

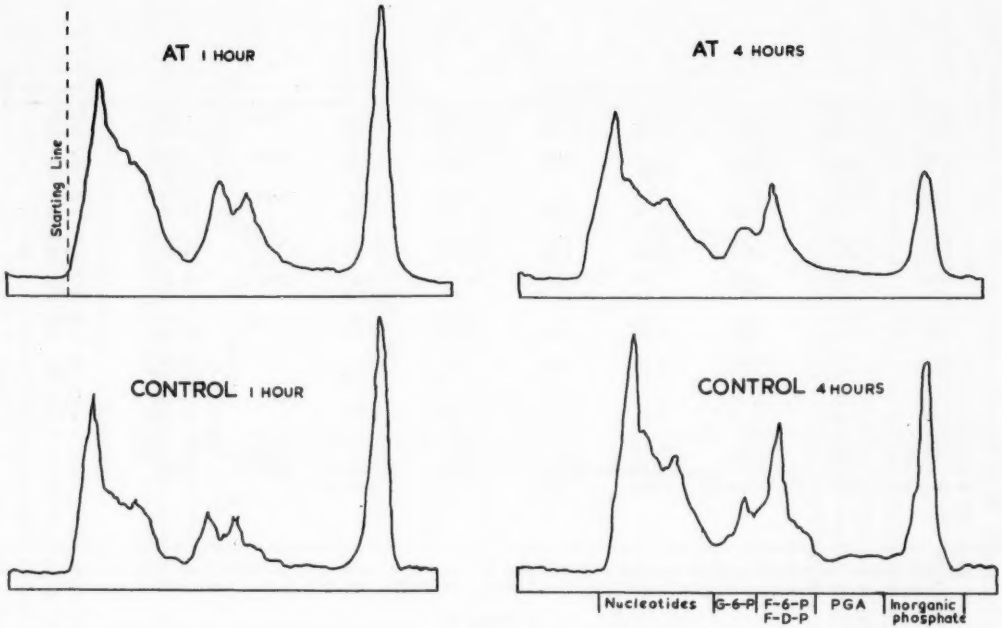

FIG. 6. The effect of AT on the incorporation of P^{32} into acid-soluble phosphate fraction of barley roots. Treatment: 48 hours in phosphate-free culture solution with or without AT (100 p.p.m.); 1 hour in 10^{-5} *M* $KH_2P^{32}O_4$; then transferred to phosphate-free culture solution. Sampled 1 and 4 hours after start of P^{32} absorption.

fraction in all plants, those to which AT had been applied contained less acid-insoluble phosphorus. At the 24th hour 74% of the P^{32} in the roots of control plants was in the acid-insoluble form but only 57% had been so fixed in AT-treated barley. The corresponding figures in the shoots were 59 and 41%. The figures show that AT-treatment resulted in a decrease in acid-insoluble fraction formation of 21.6 and 30.5% in root and shoot respectively.

The pattern of phosphorylation in both roots and shoots as revealed by the separation of the acid-soluble fraction into its three main components followed by elution and counting was found to be changed but little when the P^{32} contents were expressed as percentages of the total acid-soluble P^{32}. Figure 6 shows the close similarity between the tracings of the acid-soluble chromatogram strips of the roots of control and treated plants prepared 1 and 4 hours after the start of P^{32} absorption. Changes in the glucose-6-phosphate and fructose-6-phosphate peaks with time are also similar in the roots and in the shoots of all the plants.

The scanner records and autoradiographs of the chromatogram strips of the nucleotides and sugar phosphates further separated by the propanol/ammonia solvent also indicated that the pattern of phosphorylation was unchanged by contact with AT. The effect of AT treatment within the time of the experiment was on the metabolism of one or more of nucleic acids, phosphoproteins, and phospholipids rather than on the earlier phosphorylative steps.

Discussion

The presence of AT in barley plants caused a reduction in their ability to take up phosphate and this reduction was a function of the length of exposure to the compound. The subsequent loss of phosphate to the nutrient solution, which occurred when the plants were returned to a phosphate-free environment, was also decreased by AT treatment. This loss from the plants continued at a relatively rapid rate for a period of 3 hours and represented up to one-third of the phosphorus absorbed. The stable levels of P^{32} content were correlated with length of AT treatment. It seems reasonable to assume that, while the rapid initial loss might have been a diffusion phenomenon, the differential control of outward movement over the balance of the 96-hour period was associated with metabolism.

The content of phosphate in the barley seed is usually sufficient to supply the needs of the seedling for at least 15 days and since there were no visible signs of phosphorus deficiency in the barley plants at the time of AT application to the roots, it would appear that, unlike the situation reported by Herbert and Linck (2), where P^{32} and AT were applied to the foliage of Canada thistle, phosphate deficiency is not a prerequisite for AT inhibition of P^{32} uptake by the roots of young barley seedlings. The P^{32} content of the roots fell in all treatments whereas in the shoots the activity of only the control and 1-hour treatment decreased from the initial value. An exposure to AT of over 4 hours' duration caused a marked increase in the transport of phosphate from the root to the shoot. The steady rise in the P^{32} content of the shoots under these conditions is very different from the fluctuating amounts found in the controls. This suggests an AT inhibition of the normal downward translocation of phosphorus compounds. The 96-hour treatment with AT was the only one

after start of P^{32} absorption.

which completely prevented growth and caused the P^{32} in the shoot to fall below that of the controls. The shoot content represented over two-thirds of the total phosphate absorbed.

The translocation of absorbed phosphate to the shoots was obviously stimulated by the presence of AT, a conclusion borne out by the second experiment where the effect was even more pronounced. Under the conditions of the second experiment the 48-hour treatment with AT caused a sixfold increase in the proportion of phosphate transported to the shoot during the 1-hour absorption period and at the end of the ensuing 24-hour period this difference was still approximately threefold, i.e. 24% in the controls and 68% in the treated plants.

Although a marked increase in the amount of P^{32} in the shoot occurred, its distribution between the acid-soluble and acid-insoluble fraction was unaffected for 2 to 4 hours. The distribution of phosphate within the components of the soluble fraction was almost unchanged. The concentration of phosphate in the roots was affected by AT in a manner which suggests a correlation with the rapid conduction to the shoot. The transfer of phosphate to the fraction containing nucleic acid, phosphoprotein, and phospholipid was considerably reduced by AT and this may well have resulted in a diversion of the balance of phosphate to the system responsible for upward movement. It has been shown (5) that a number of metabolic inhibitors, while decreasing total uptake, cause an increased transfer of phosphate to the shoot and in this latter respect they resemble AT.

More detailed examination of the acid-soluble fraction revealed the interesting fact that the pattern of phosphorylation was virtually unaffected by treatment with AT. In this respect the effect of AT differs from that of 2,4-dinitrophenol (DNP) and iodoacetate. Whether the latter compounds uncouple phosphorylation from oxidative metabolism, e.g. DNP (5), or whether the effect is at substrate level as in the case of iodoacetate (4), great differences are seen in the phosphorylative pattern within the tissue. DNP drastically reduces the incorporation of phosphate into the nucleotide fraction, but has virtually no effect on the proportion of the absorbed phosphate entering the nucleic acid fraction.

It appears that the effect of AT on phosphorus metabolism is not concerned with oxidative phosphorylation or with the normal glycolytic pathway, but is confined to the processes involved in the incorporation of phosphate into the components of the acid-insoluble fraction (nucleic acids, phosphoproteins, phospholipids). The intimate connection between nucleic acid synthesis and protein formation in living tissues suggests the possibility that a major effect of AT in plant metabolism could be interference with normal synthesis of protein. An altered phospholipid metabolism might have profound effects upon chloroplast structure.

It has been reported that AT applied to leaves inhibits the development of plastids and causes a reduction in chlorophyll synthesis (8). The esterification pattern in the leaves of normal plants reflects the presence of both oxidative and photosynthetic phosphorylation. The fact that the utilization of phosphate in the leaves of both control and treated plants is identical suggests that the portion of the photosynthetic mechanism associated with incorporation of inorganic phosphate into organic forms is in no way affected by AT.

References

1. FREDRICK, J. F. and GENTILE, A. C. 1959. The effect of amino triazole on algal phosphorylase. Plant Physiol. **34**, (Suppl.) XIV.
2. HERBERT, R. A. and LINCK, A. J. 1958. Studies on the role of phosphorus in the action of 3-amino-1,2,4-triazole. Abstr. Weed Soc. Am. 36–37.
3. LOUGHMAN, B. C. and MARTIN, R. P. 1957. Methods and equipment for the study of the incorporation of phosphorus by intact barley plants in experiments of short duration. J. Exptl. Botany, **8**, 272–279.
4. LOUGHMAN, B. C. and MIETTENEN, J. K. Unpublished.
5. LOUGHMAN, B. C. and RUSSELL, R. S. 1957. The absorption and utilization of phosphate by young barley plants. IV. The initial stages of phosphate metabolism in roots. J. Exptl. Botany, **8**, 280–293.
6. MCWHORTER, C. G. 1959. Some effects of 3-amino-1,2,4-triazole on the respiratory activities of Zea mays. Dissertation Abstr. **19**, 1529.
7. MILLER, C. S. and HALL, W. C. 1957. Effects of amino triazole salts and derivatives on cotton defoliation, growth inhibition, and respiration. Weeds, **5**, 218–226.
8. PYFROM, H. T., APPLEMAN, D., and HEIM, W. G. 1958. Catalase and chlorophyll depression by 3-amino-1,2,4-triazole. Plant Physiol. **32**, 674–676.
9. ROGERS, B. J. 1957. Chlorosis in corn as induced by the herbicide 3-amino-1,2,4-triazole. Plant. Physiol. **32**, (Suppl.) VI.
10. RUSSELL, J. 1957. Amino triazole interactions in the growth of timothy grass seedlings. Can. J. Botany, **35**, 409–434.
11. RUSSELL, R. S. and MARTIN, R. P. 1953. A study of the absorption and utilization of phosphate by young barley plants. I. The effect of external concentration on the distribution of absorbed phosphate between roots and shoots. J. Exptl. Botany, **4**, 108–127.
12. SUND, K. A. 1960. Reduction of 3-amino-1,2,4-triazole phytotoxicity in tomato plants. Agr. Food Chem. **8**, 210–212.
13. WORT, D. J. and SHRIMPTON, D. M. 1958. Effect of foliar applied amino triazole on the photosynthesis and respiration of leaves of bean and wheat, and on cytochrome oxidase activity in wheat. Research Rept. National Weed Committee, Western Section. Can. Dept. Agr. 124–127.

LYCOPERDON IN EASTERN CANADA WITH SPECIAL REFERENCE TO THE OTTAWA DISTRICT[1]

CONSTANCE A. BOWERMAN

Abstract

Twenty-one species of the genus *Lycoperdon* have been described, one of which is a new species, *L. flavotinctum*. Eighteen species have been found in the Ottawa area while the remaining three could be expected to occur.

Introduction

Although the Gasteromycetes have been studied more or less intensively in the United States by several authors including Morgan (1891), Coker and Couch (1928), Kambly and Lee (1936), Smith (1951), and others, very little has been published with reference to their occurrence in Canada. Accordingly a study of the Gasteromycetes of the Ottawa District was undertaken. This includes the area within a 30-mi radius from Ottawa, and the species occurring here are probably representative of eastern Canada in general. Species of the genus *Lycoperdon* proved to be the most common members of this group and this paper presents a summary of the *Lycoperdon* species found in this area.

Preliminary studies indicated that about six species occurred here but with more intensive collecting the number has been increased to 18, of which one is described as new. Three other species which might be expected to occur here have also been included. *L. echinatum* Pers. has been found in more northerly areas as well as farther south, and *L. floccosum* Lloyd and an immature specimen of *L. pulcherrimum* B. & C. have been found near Kingston, Ontario.

In the nomenclature, an effort has been made to follow the International Code and in this respect the work of Perdeck (1950), who recently studied the specimens in the Persoon Herbarium, has been invaluable.

The species of *Lycoperdon* can readily be divided into two groups, the first, the smaller of the two, consisting of those species with a purplish-brown gleba at maturity and distinctly rough spores at a magnification of ×440, the second consisting of those species with an olive-brown gleba at maturity and smooth or slightly rough spores at a magnification of 440. Occasionally some species of the second group may also show a purplish cast if the fruit bodies are old and weather beaten; therefore both characteristics should be noted in separating the two groups. Within these groups the characters of the cortex and spores are used to distinguish the species.

Lycoperdon Pers.

Basidiocarp small to medium-sized, 1–10 cm in diameter, permanently attached to the substratum, globose, depressed-globose, obovoid, pyriform

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to turbinate, some sessile and some with a distinct stalk; cortex, which usually falls off at maturity, consisting of warts, spines, scales, and granules; inner peridium thin, membranous, surface usually smooth or with distinct markings, opening by a distinct apical pore; gleba white at first but becoming yellowish to olive-brown or purple-brown at maturity; subgleba usually present, cellular or compact; capillitium of long threads, slender, simple or branched, thickness of threads similar to that of spores, branches tapering; spores small, globose, rarely oval or elliptic, smooth, spiny or warted, sessile or with a minute pedicel.

This genus is distinguished from *Calvatia* by the presence of an apical pore, and from *Bovista* by the firm attachment of the fruiting body to the substrate.

The fruit bodies are found mostly on the ground on earth or on rotten wood, occasionally among moss or on the bark of living trees. The basidiocarps range in size from very small up to about 4–5 cm, occasionally up to 6 cm in diameter or height. They may be found growing in large groups as in *L. pyriforme* Pers. but more commonly occur singly, or two or three may form a small cluster.

Key to the Species

1. Gleba with a purplish cast at maturity; spores distinctly rough at a magnification of 440 . . 2
1. Gleba olive-brown at maturity; spores smooth or slightly rough at a magnification of 440 . 9
2. Cortex felty, becoming rimose at maturity; spores warty, 5.5–7.5μ in diameter . . . . . *L. rimulatum*
2. Cortex otherwise 3
3. Cortex composed of long slender spines 4
3. Cortex composed of small spines or warts, or of spines and granules 6
4. Spines pale at maturity and, on falling off, leave the peridium smooth . . *L. pulcherrimum*
4. Spines dark-colored at maturity and on falling off leave the peridium reticulate . . . . . *L. echinatum*
5. Cortex appearing granular 6
5. Cortex otherwise 7
6. Cortex of small persistent granules; spores 5.5–6μ (including spines) . . . . . *L. glabellum*
6. Cortex of granules and small spines; spores 6.5–8μ (including spines) . . . . . *L. delicatum*
7. Spores rough at a magnification of 440 8
7. Spores smooth at a magnification of 440; cortex of dark-colored short spines, or spines and granules; spores 4–5μ in diameter . . . . . *L. umbrinum*
8. Cortex a flocculent covering of spines and warts; spores 5.5–6μ (including spines) . . . . . *L. floccosum*
8. Cortex of brownish to white, slender spines and granules; spores 5–6μ (including spines) . . . . . *L. molle*
9. Subgleba present 10
9. Subgleba absent 19
10. Subgleba cellular 11
10. Subgleba compact 18
11. Cortex composed of distinct spines or warts 12
11. Cortex composed of granules, or small spines and granules 15
12. Basidiocarp white or cream at maturity 13
12. Basidiocarp cream to olive-brown at maturity 14
13. Warts flaking off in groups revealing a dark inner peridium; capillitium yellow-brown . . . . . *L. candidum*
13. Spines or warts falling off revealing a paler inner peridium; capillitium hyaline . . *L. curtisii*
14. Spores pedicellate, subglobose to oval . . . . . *L. pedicellatum*
14. Spores without pedicels, globose . . . . . *L. perlatum*

15. Basidiocarp cream to olive-brown at maturity....16
15. Basidiocarp reddish brown at maturity....17
16. Cortex composed of granules with a few small spines or of simple spines, spores 4–4.5 μ in diameter....*L. muscorum*
16. Cortex composed of floccose granules at first, becoming aggregated to form small spinulose granules; spores 4–5 μ in diameter....*L. spadiceum*
16. Cortex composed of pale brownish, minute spines convergent at their tips, or of separate spines and granules; spores 4–4.5 μ in diameter....*L. flavolinctum*
17. Spores smooth, 3.5 μ; surface of fruiting body rough to the touch like a file..*L. pyriforme*
17. Spores spiny, 4–5 μ; surface of fruiting body becoming pitted at maturity....*L. subincarnatum*
18. Basidiocarp usually yellowish with small floccose warts or granules; spores 4–4.5 μ in diameter....*L. coloratum*
18. Basidiocarp brownish olive with granules and warts or spines; spores 3.5–5 μ in diameter....*L. ericetorum*
19. Basidiocarp up to 1 cm high, spores smooth, 3.5–4.5 μ in diameter....*L. acuminatum*
19. Basidiocarp up to 2 cm in diameter, spores smooth to minutely spiny, 4–5 μ in diameter....*L. pusillum*

Lycoperdon acuminatum (Bosc.) Fries, Novae symbolae 118. 1851.

Basidiocarp small, 8 mm broad and 10 mm high, obovoid at maturity, with a small fibrous root, somewhat plicate at the base; drab (R),[2] opening by a prominent apical pore with a lobed edge; cortex composed of whitish, floccose granules and furfuraceous material which gradually wears away revealing the darker (Saccardo's umber (R)), shiny, almost smooth, inner peridium; gleba olive-brown; subgleba absent; capillitium pale yellowish brown with distinct, but thin walls containing a few minute pits, only occasionally branched, tapering and slightly sinuous, 2.5–5 (6.5) μ in diameter; spores globose, concolorous with the capillitium, usually with a minute pedicel, 3.5–4.5 μ in diameter, appearing smooth but at a magnification of 950, seeming to be surrounded by a hyaline envelope into which some very minute spines project.

Habitat: Among moss in an open field.

Specimens examined: DAOM 50008, Merivale Road, Ont.; BPI, Lloyd 30566.

Only a single specimen of *L. acuminatum* has been collected in this area. The species is distinguished by its extremely small size and obovoid shape, by the absence of a subgleba, and by the spores, which seem to be very minutely spiny at a magnification of 950. *L. acuminatum* has previously been reported as occurring on moss-covered bark of living trees at a height of 4–15 ft, on old mossy logs or mossy bases of living trees, but even though this specimen was found in moss in an open field its characteristics were unmistakable.

A collection of this species (Lloyd 30566) was examined in the Lloyd Herbarium which had been collected by A. P. Morgan at Preston, Ohio, and determined by C. G. Lloyd. The fruit bodies in this collection were found to agree both macroscopically and microscopically with our specimen except for the brighter color of the gleba in Lloyd's fruit bodies, but this could well be due to a difference in maturity. The capillitium was found to be 3.5–5.0

[2]Colors are from Ridgway, R. Color standards and nomenclature. Washington. 1912.

(5.5) μ in diameter, rarely branched, thin-walled with minute pits, while the spores were found to be 3.5–4.5 μ in diameter, globose with a minute pedicel, smooth to faintly dotted or minutely spiny at a magnification of 950.

Lycoperdon candidum Pers., Syn. Fung. p. 146. 1801. non *L. candidum* (Rostk.) Bon. 1888. (Fig. 2).

Lycoperdon cruciatum Rostk., *in* Sturm, Deutschl. Fl. 3: fasc. 18, p. 19. 1839.
Lycoperdon marginatum Vitt., Mon. Lyc. p. 41. 1842.
Lycoperdon calvescens Berk. & Curt., Grevillea, 2: 50. 1873.
Lycoperdon separans Peck, Ann. Rep. N.Y. State Museum 26: 73. 1874.
Lycoperdon papillatum Schaeff. ex Hollos, Gastr. Ung. p. 108. 1904.

Basidiocarp 1.0–4.5 cm broad, 1.0–2.8 cm high, usually broader than tall, globose to subglobose at first but becoming very much depressed, plicate on the under side, narrowing abruptly to a point of attachment which usually extends to a basal rhizomorph; cortex white, composed of either coarse warts with sharp tips or of compound warts with the tips convergent, warts 1–2 mm long above, smaller and thinner downward, falling off above in groups, rarely singly, exposing a velvety-tomentose to furfuraceous, buffy brown (R) to olive-brown (R) layer which may appear reticulate; cortex persistent below, but usually becoming discolored; tomentose layer gradually wearing away revealing the paler, shiny, inner peridium; apical pore formed after the exoperidium has fallen off, up to 5 mm in diameter; gleba white at first, changing to yellow and finally buffy olive (R) to olive-brown (R); subgleba white at first, changing to olive-brown at maturity, occupying the basal portion or up to one-half of the basidiocarp extending up the sides and sharply separated from the gleba, chambered; capillitium, when mature, a medium yellow-brown color, with distinct darker-colored walls up to 0.8 μ thick, occasionally pitted, sinuous in the thinner portions, irregular thickenings along the edge, branched, occasionally septate, tapering both gradually and abruptly, variable in diameter (1.6) 3.5–5.0 (8) μ in diameter; spores globose, slightly lighter in color than the capillitium, some with small pieces of pedicels attached, smooth or slightly rough under oil, surrounded by a thin, hyaline envelope, pieces of hyaline pedicels mixed with the spores, 3.5–4.0 μ in diameter.

Habitat: on sandy ground in woods or in pastures. June–October.

Specimens examined in DAOM: 27419, 27443, 27452, 27453, 27972, 29450, 29459, 29491, 45090, Bell's Corners, Ont.; 22187, Constance Bay, Ont.; F 9562, Horse Shoe Bay, Ont.; 22168, Fitzroy Harbour, Ont.; 29479, Kingston, Ont.; 22140, Lake Opinicon, Ont.; 27405, 27447, 27979, 29483, Merivale, Ont.; 27432, North Gower, Ont.; 22239, Petawawa Forest Experiment Station, Ont.; 7797, Port Alexander, Ont.; 16772, Eardley, Que.; 26405, Sand Bay, Que.; 7791, Digby, N.S.; 39420, Kentville, N.S.; 27397, Devereux Lake, Mich.,

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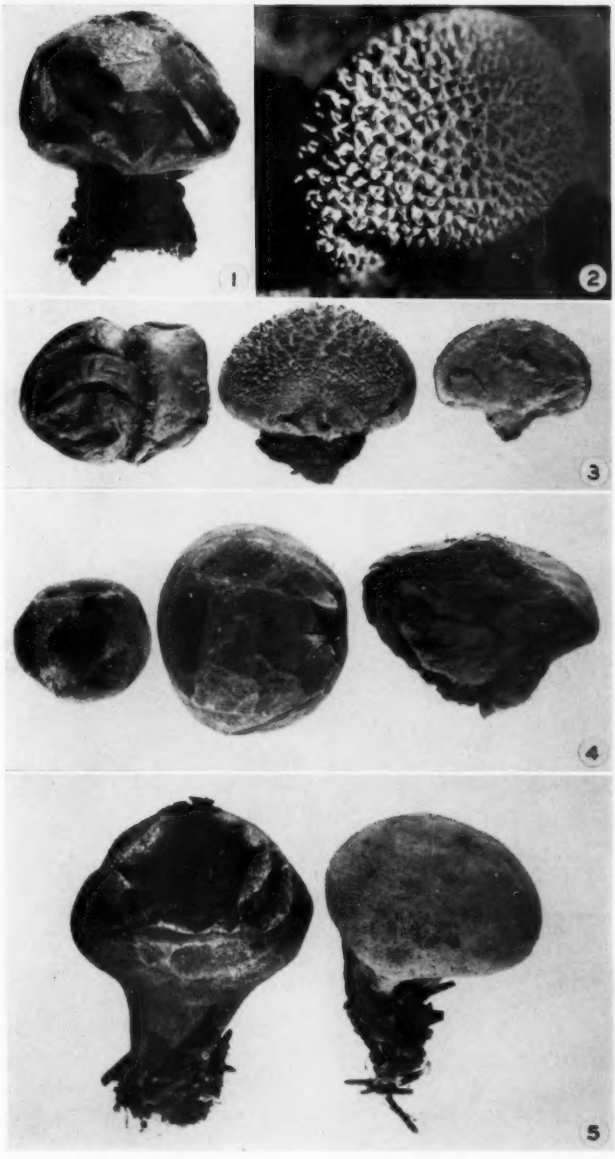
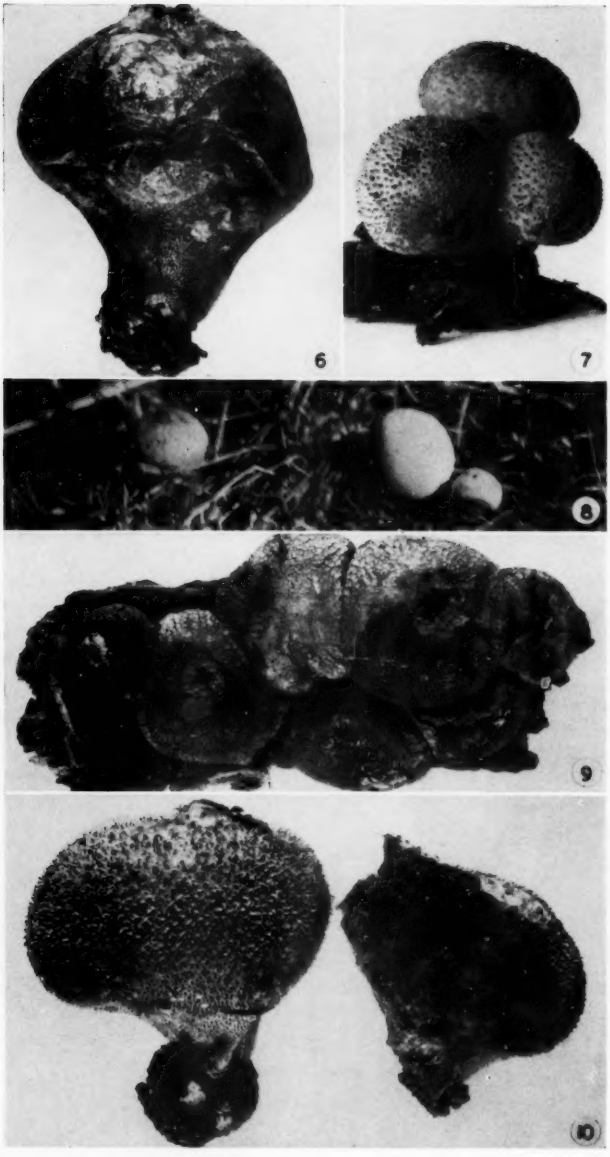
FIG. 1. *Lycoperdon coloratum*, ×1.3 approx. Fig. 2. *L. candidum*, ×2 approx. Fig. 3. *L. curtisii*, ×1.3 approx. Fig. 4. *L. flavotinctum*, ×1.3 approx. Fig. 5. *L. molle*, ×1.3 approx. Fig. 6. *L. umbrinum*, ×1.3 approx. Fig. 7. *L. pyriforme*, ×1 approx. Fig. 8. *L. pusillum*, ×1.5 approx. Fig. 9. *L. subincarnatum*, ×1.3 approx. Fig. 10. *L. pedicellatum*, ×1.3 approx.

PLATE I



Bowerman—Can. J. Botany

PLATE II



Bowerman—Can. J. Botany

U.S.A.; 24743, Ithaca, Mich., U.S.A.; 26790, Idaho Co., Idaho, U.S.A. (Wm. Bridge Cooke); 24831 ex FH Richmond, Virginia, U.S.A.

L. candidum Pers. is a common species in the Ottawa area and is found on the ground, usually in open places. It is readily recognized by the coarse white warts which soon fall off in patches, revealing a dark, tomentose to furfuraceous layer which, in turn, wears away gradually to expose the paler, shiny inner peridium. The spores are mixed with pieces of hyaline pedicels and are globose, smooth, or slightly roughened due to the presence of extremely small spines, which can barely be seen at a magnification of 950.

The immature stages of *L. candidum* and *L. curtisii* Berk. are similar in appearance and could be confused macroscopically. However, *L. candidum* is usually of a larger size and lacks the hyaline or almost hyaline capillitium and smaller, 3–3.5 μ, echinate spores of *L. curtisii*.

This species has been commonly known as *L. marginatum* Vitt. and less commonly as *L. cruciatum* Rostk. in North America, but according to Perdeck (1950), these names are synonyms of *L. candidum* Pers. Our collections seemed to fit the description Perdeck made from Persoon's material except no mention was made in his description of any roughness on the mature spores or of pieces of hyaline pedicels mixed with the spores. A slide from the type of *L. candidum* Pers. (HLB 910, 258–497) was examined and the spores appeared to be immature and were found to be smooth, globose to subglobose or pyriform, 2.5–3.5 μ in diameter with long pedicels still attached to the spores. The characteristics of the spores from this slide did not seem to agree with those described by Perdeck, which were said to be "3.5–4 μ in diam. globose, smooth;".

However, through correspondence with Perdeck and Maas Geesteranus, the following information concerning the type specimen was obtained. "1. The fine preservation of the exoperidium of the fungus suggests a juvenile specimen. 2. The great majority of the spores was found in an early stage of development, medium sized spores were few, mature spores very few. 3. The mature spores cannot be considered impurities from alien source, since several intergradations were found. 4. Immature spores, seen in lactophenol, are pale yellow, globose to pyriform, perfectly smooth, with short to very long pedicels (up to 7 and 9μ), sometimes also without a pedicel, measuring 2.7–3.6 μ. Intermediate spores 3.6–4.5 μ, are of the same color, possess a short pedicel or none at all, whereas in some of them the spore wall starts to develop tiny spines. Mature spores cinnamon colored, globose, 4.5–5.4 μ (1.5–2 μ less if the spines are not counted), (as far as seen) devoid of pedicel, minutely warty with spiny excrescences." Therefore according to Perdeck and Maas Geesteranus "1. pedicels do occur, though in immature spores only; 2. mature spores are minutely warty, not smooth". It is evident that the slide from the type material contained immature spores which agree with the characteristics of the immature spores given above. Since the Persoon material is immature, there would be few, if any, pieces of hyaline pedicels in the mounts and these could be easily overlooked. The pedicels on the immature spores are certainly long enough and of the correct width to account for the presence of pedicels in the mounts of mature spores in our collections.

The characteristics of the spore wall still seem to differ from those of the type, as our mature spores are only very minutely spiny and are surrounded by a hyaline envelope which is about as thick as the length of spines in the type. In the Canadian collections, when other characteristics have been the same, the spores examined at a magnification of 950 were found to be perfectly smooth in some, minutely warty in others, or both smooth and minutely warty in still others. The variable character of the spore wall in this species thus appears to be a result of its very slow development to full maturity. Lohman (1927) described the spores as smooth, but found some specimens with rough spores, almost echinulate, which he placed in *L. separans*. Smith (1951) found the walls of the spores in *L. marginatum* to be minutely punctate from pores in the walls, while Coker and Couch (1928), Kambly and Lee (1936), and Hollos (1904) state that the spores are smooth.

In spite of the difference of opinion and uncertainty concerning the character of the spore wall, I feel that our species is best disposed as *L. candidum* Pers.

Lycoperdon coloratum Peck, N.Y. State Mus. Rep. 29:46. 1878. (Fig. 1)

Basidiocarp subglobose or obovate, 9–17 mm high and 8.5–17 mm broad, with a white, slender, cord-like root attaching it to the substratum, ochraceous buff (R) to cinnamon buff (R) to tawny olive (R) at first, but darkening to buffy brown (R) above with the paler colors at the base; cortex composed of small floccose warts or granules which become aggregated into small groups which wear away revealing the somewhat paler, shiny, inner peridium, or rarely of small warts which separate as the basidiocarp matures and gradually become floccose especially near the base; opening by a central apical pore up to 3–4 mm in diameter; gleba close to honey yellow (R) but becoming buffy brown (R) to light brownish olive (R) at maturity; subgleba concolorous with the gleba, small, occupying up to one-quarter of the basidiocarp, compact; capillitium pale yellowish in the yellow specimens but becoming yellow-brown in the brown specimens with distinct walls up to 0.7 μ thick, very much branched, tapering gradually, some very thin threads, but usually (2.5) 3.5–4 (6.5) μ in diameter; spores almost concolorous with or lighter than the capillitium, globose, with a minute pedicel, some smooth and some minutely spiny at a magnification of 950, (3.5) 4–4.5 μ in diameter.

Habitat: on the ground, usually singly, Aug. to Oct.

Specimens examined in DAOM: 27413, 27414, 27970, North Gower, Ont.; 29482, Western Lookout, Gatineau Park, Que.; 28002, Pellston Hills, Michigan, U.S.A.; 27973, Tahquamenon Falls, Michigan, U.S.A.

The above description was made mainly from the type, which was collected by C. H. Peck, at Sandlake, N.Y. This collection consisted of four fruit bodies pasted to pieces of cardboard making it difficult to determine their true size and shape. Three of the fruit bodies were intact while the fourth had been cut longitudinally through the center revealing the yellowish gleba and the small, compact, concolorous subgleba. The fruit bodies were found to be subglobose, 10–17 mm high and 8.5–16 mm broad, with a slender, cord-like root and ochraceous buff (R) to cinnamon buff (R) in color. The cortex is

composed of small yellowish granules (similar to those of *L. pusillum*) which appear floccose and separate somewhat to reveal the paler, slightly shiny, inner peridium. The capillitium was found to be pale yellowish with distinct walls up to 0.7 μ thick, very much branched, tapering, (2.5) 3.5–4 (6.5) μ in diameter, and the spores to be concolorous with the capillitium, globose with a minute pedicel, smooth or some minutely spiny at a magnification of 950, (3.5) 4–4.5 μ in diameter. These characteristics agree well with those in the original description, but Peck (1879) later described more mature fruit bodies where he describes the cortex as becoming brownish when old and the subgleba, a dingy olive. Also in the discussion at the end of this description, the immature plant is said to be quite conspicuous while the mature plant is said to be quite inconspicuous as it closely resembles the brown color of the fallen leaves.

The collections from North Gower were old and were a dark brownish color with a small amount of yellow at the base. The cortex was of the same structure as that in the type but the capillitium was slightly darker in color, and a very small compact subgleba was present. Peck (1879) made no reference to the presence of a subgleba in his description even though one was present in the type.

In the Michigan and Quebec collections, the fruit bodies are yellowish but very immature and the cortex is composed of small, slightly darker tipped warts which become separated, especially at the base in these specimens, where the cortex is composed of floccose granules similar to those in the type. Thus it would seem that the warts gradually break up as the plant matures to become floccose granules. Also weather conditions are known to cause considerable variation in the structure of the exoperidium.

From the presence of the compact subgleba in the fruit bodies, this species seems to be closely related to *L. ericetorum* and could very well be a color form of this species. However, until more collecting and study can be done with this group, it seems advisable to retain *L. coloratum* Pk. as a separate species.

Lycoperdon curtisii Berk., Grevillea, 2: 50. 1873. (Fig. 3).

Lycoperdon wrightii Berk. & Curt., Grevillea, 2: 50. 1873.

Basidiocarp 6–15 (20) mm high and (5) 8–18 (30) mm broad, globose, subglobose to depressed globose, appearing sessile but with a short, tapering base, plicate beneath, attached to the ground by a few white rhizomorphs, white to cream-colored; cortex composed of warts or more usually of spines about 1–2 mm long, united at their tips with divided bases, with groups of spines converging at their apices to form stellate groups, becoming smaller downward and finally granular at the base, often with fine furfuraceous material between the spines which increases in amount downward; spines falling off revealing a farinaceous, chamois (R) to dark olive-buff (R) inner peridium which gradually becomes smooth and shiny; pore apical, slow to form, small, usually 2–3 mm in diameter, gleba white at first but becoming yellow-brown and finally a dark olive-buff (R) at maturity, usually with a small columella; subgleba white at first and remaining almost white in some, while in others it becomes concolorous with the gleba or develops a purplish

cast at maturity, chambered, convex above, distinctly separated from the gleba, occupying up to ⅓ to (½) of the basidiocarp; capillitium hyaline, thin-walled, septate, sparsely branched, a few irregular thickenings along the edge, with much debris adhering, (3.5) 5–6.5 (11.5) μ in diameter; spores globose, pale brownish yellow, some with a small pedicel, smooth or very minutely roughened at a magnification of 440, minutely spiny at a magnification of 950, (2.5) 3–4 μ in diameter.

Habitat: solitary or caespitose, on sandy ground, among grass and occasionally on compost heaps. Aug. to early Oct.

Specimens examined in DAOM: 27988, 27994, 29464, 29470, 29472, 29500, 45079, 45109, 45121, Bell's Corners, Ont.; 29490, Constance Bay, Ont.; 22180, Fitzroy Harbour, Ont.; 25796, 25808, Lake Opinicon, Ont.; 22152, 22163, 22182, 22185, 22210, 22213, 22217, 25794, 25806, 39417, Central Experimental Farm, Ottawa, Ont.; 7789, 22203, Ottawa area, Ont.; 29481, Kingsmere, Que.

This is a very attractive little puffball which usually grows in small groups or clusters and is common in late summer and fall in pastures, lawns, and occasionally along roadways. Macroscopically the cortex resembles that of *L. candidum* Pers., but *L. curtisii* is readily separated from *L. candidum* by its smaller size, the more persistent cortex, which does not flake off in large patches, the paler inner peridium, and the larger, thin-walled, hyaline, septate capillitium, as opposed to the darker capillitium of the latter. Both *L. curtisii* and *L. subincarnatum* Pk. have a hyaline capillitium, but here the resemblance ends as *L. subincarnatum* has a pinkish brown cortex and a pitted inner peridium.

L. depressum Bon. has a hyaline, septate, thin-walled, flaccid, mostly granular, sparingly branched capillitium up to 5.6 (8.1) μ in diameter and this is very similar to that of *L. curtisii*. However, according to Perdeck (1950), the fruit bodies are 1–5 cm high and 1.5–6 cm broad with a variable height–width ratio and only sometimes sessile, globose, or depressed globose, whereas our specimens are small and have all been globose to depressed globose. Also in *L. depressum*, the surface is composed of fairly fugaceous white or yellowish spines which are very small and often clustered and connivent, or sometimes with mealy granules, or small scales, and the gleba is without a columella and separated from the subgleba by a diaphragm. Therefore *L. curtisii* is very close to *L. depressum* except for the smaller size of the fruit bodies and the larger and longer warts and spines on the cortex. Although there is a sharp separation between gleba and subgleba in *L. curtisii*, there is no evidence of a distinct diaphragm being present as in *L. depressum*.

There has been considerable confusion about the correct name for this fungus. If *L. curtisii* and *L. wrightii* are the same species, *L. curtisii* is the correct name as it was described as number 333 while *L. wrightii* was described by Berkeley and Curtis as number 334. With these facts in mind, Dr. J. W. Groves kindly examined the types of both of these species in the Kew Herbarium and made notes on the specimens in 1954. From the notes the following information has been obtained.

In the type folder of *L. wrightii* B. & C., there are two specimens from Berkeley's herbarium, one from New Jersey and the other from Connecticut. Since the Connecticut specimen bears the number 5633, was collected by C. Wright, and is cited in the original description, it should be considered as the type of the species. This collection consists of three specimens glued to a sheet of paper and these were "somewhat badly flattened but appear to have been nearly globose and are 1 to 1.5 cm in diameter." The outer peridium consisted of whitish buff, conical spines up to 1 mm long above and about 0.2 mm below which are very deciduous, and the inner peridium was smooth. There did not seem to be a columella but the subgleba was about 1 mm thick, concolorous with the gleba and "apparently sharply separated from the gleba."

There were four collections mounted on one page in a type folder of *L. curtisii* B. A 1938 collection from Bermuda and another (2195) labelled "*Lycoperdon.* Car. Inf." can be disregarded as they are not cited in the original description. Another specimen labelled 5613=558 *Lycoperdon curtisii* B. Connecticut, Wright, consisted of two fruit bodies glued to a sheet and two loose fruit bodies in a small packet and was cited in the original description. The last specimen, labelled 558 Car. Sup. *Lycoperdon curtisii* B., M. A. Curtis, was also cited in the original description. It is the first collection cited and is collected by Curtis and so should be taken as the type of the species. This collection consisted of nine fruit bodies glued to a sheet, four and part of a fifth still remain, and fragments of at least two others are in two small packets. The fruit bodies are "from 5–8 mm. in diameter but one in the Connecticut specimen is 10 mm. in diameter" and the "exoperidium consists of pointed spines tending to come together at the tips to form pyramidal warts". There was not much evidence of the exoperidium being deciduous in the Curtis specimen, but the fruit bodies seemed to be immature. However, in the larger specimen of the Wright collection, the spines "are falling off and are more evident as separate spines. In this specimen they look quite like the spines in *L. wrightii.*"

Mounts were also made from the type specimens mentioned above and they were found to be almost identical. In a mount of *L. curtisii*, the spores were found to be (3) 3.5 μ in diameter, minutely spiny at a magnification of 950, hyaline with only a few, pale yellowish; the capillitium was found to be up to 7 μ thick, thin-walled, hyaline, and septate, while in a mount from *L. wrightii*, the spores were found to be (3) 3.5 (4) μ in diameter, minutely spiny at a magnification of 950, and pale yellowish, and the capillitium to be up to 8 μ thick, thin-walled, hyaline, septate, and somewhat granular.

From this it would appear that the two fungi are very close if not identical. The main microscopic difference between them is that the capillitium of *L. wrightii* is granular and somewhat broader than that in *L. curtisii*, and the spores in *L. wrightii* have a yellowish color. However, the mount of *L. curtisii* is made from slightly immature material as the spores are still hyaline and the capillitium appears immature. Macroscopically the two species are also very similar and it would be difficult to tell them apart. *L. wrightii* seems to be larger with the smallest specimen being 1 cm in diameter, whereas the largest specimen of *L. curtisii* just reached 1 cm in diameter. "However from

the gleba and the outer peridium, it is evident that all the specimens of *L. wrightii* are more mature than those of *L. curtisii*." The same difference in size has been found in collections from the Ottawa area and there have been collections with specimens of both sizes; also the differences in the capillitium have been found in the same collection and sometimes in the same fruit body, depending on where the mount was made. Therefore, in my opinion, *L. curtisii* is the correct name for the fungus and *L. wrightii* is a synonym.

Coker and Couch (1928), rightly considered *L. wrightii* to be a synonym of *L. curtisii* whereas Lloyd took *L. wrightii* to be a valid name. He stated that "*Lycoperdon curtisii* is based on some young specimens of a larger species (probably *cruciatum*) just emerged from the ground and should never have been determined, much less named and described". However, this species has not the characteristics of *L. cruciatum* (*L. candidum*). Referring to *L. wrightii*, Lloyd also stated that "The species was named and described by Berkeley from some old specimens that had lost their cortices and he gave a fairly good description of it excepting he mistook the furfuraceous remains of an old peridium for the cortex and described it as having 'minute echinulate warts' ". Morgan (1891) and Kambly and Lee (1936) recognize both a *L. curtisii* and a *L. wrightii*, and their *L. curtisii* is the same as the species described here, but what their *L. wrightii* is I do not know.

Lycoperdon delicatum Berk. sensu Morgan, Journ. Cinn. Soc. Nat. Hist. 13:10. 1891.

Basidiocarp 2.5 cm tall, subglobose with a root-like projection and some white mycelial fibers at the base, plicate below; isabella color (R) to honey yellow (R) above, chamois (R) to cream buff (R) below; cortex somewhat wrinkled, composed of small granules and fine spines which wear away above, giving the peridium a granular appearance, almost smooth to the naked eye, granules quite persistent, only rarely revealing the smooth, shiny, inner peridium; pore apical; gleba with a small pseudocolumella, olive-brown with a purplish tint; subgleba cellular, cream-colored, small, occupying one-fifth of the basidiocarp or the small, tapered portion at the base; capillitium pale yellowish with a distinct, unpitted wall up to 0.7 μ (occasionally 1.4 μ) thick, only occasionally branching and usually at a sharp angle, irregular thickenings along the edge, contorted, sinuous in the thinner portions, tapering gradually or rather abruptly, 3.5–5.5 μ in diameter; spores concolorous with the capillitium, globose with a short piece of pedicel still attached, long pieces of hyaline pedicels mixed with the spores, rough at a magnification of 440 but spiny at a magnification of 950 with spines up to 1 μ long projecting into a hyaline envelope, 6.5–8 μ in diameter including the spines (5–6 μ in diameter without the spines).

Habitat: on the ground. Sept.

Specimen examined in DAOM: 28010, Gatineau Park, Que.

The exact identity of *L. delicatum* is in doubt. Massee (1887) included in his monograph a specimen from the Khasia Mountains in the northwest Himalayas which he called *L. delicatum* Berk. and which he described as having

echinulate spores about 6 μ in diameter. This is apparently based on *L. delicatum* Berk. described in Hook. Journ. Bot. 1854, p. 172. It seems unlikely that this is the same fungus as *L. delicatum* Berk. & Curt. in North Amer. Fung. 337, which was said to have small, smooth spores about 4 μ in diameter.

Morgan (1891) applied the name *L. delicatum* Berk. to a fungus which appeared to agree with Massee's (1887) description in spore characters but differed in having the capillitium only occasionally branched. Our specimen appears to agree with Morgan's concept but only examination of the type material can determine whether or not it is the true *L. delicatum* Berk.

Our species is characterized by its nearly smooth surface and large, spiny spores. The surface of the basidiocarp appears to be smooth and wrinkled to the naked eye but under a lens small spines are seen to be present on the lower part and small granules on the upper part. Morgan (1891) described the basidiocarps as 1–2 in. (2.5–5 cm) in diameter and the single specimen found here was 2.5 cm.

Morgan (1891) described the subgleba as being "very small or quite obsolete" and the capillitium as "rather thinner than the spores". Both of these characters apply in the Gatineau Park specimen and it is thereby excluded from *L. elegans* Morg., which has a fairly large subgleba and a capillitium thicker than the spores, and from *L. molle* Pers., which has a large subgleba and a capillitium which averages the same size as the spores. Hollos (1891) gave essentially the same description as did Morgan (1891) but treated the fungus as var. *delicatum* Morg. of *L. umbrinum* Pers.

Lycoperdon echinatum Pers., Syn. Meth. Fung. 147. 1801.

Lycoperdon constellatum Fr., Syst. Mycol. 3: 39. 1829.

Basidiocarp 2.2–3.5 cm high and 2.5–3.5 cm broad, subglobose with a short stout stalk or broadly pyriform with fine white rhizomorphs at the base penetrating the substratum, plicate beneath, Saccardo's umber (R) above and tawny olive (R) below; cortex composed of slender spines 2–3 mm long, white at first but soon becoming brownish, darker at the apex, with tips convergent or a number of spines joined together at the base and these groups convergent at their apices, falling off leaving a reticulated inner peridium composed of furfuraceous material and small warts which may wear away very quickly, leaving a pale to dark, smooth, shiny, inner peridium; pore apical, very slow to form; gleba white when young, but changing from light to dark brown and finally with a distinct purplish tinge at full maturity; subgleba about the same color as the gleba, composed of very small cells, usually small but sometimes occupying up to ½ of the basidiocarp; capillitium light yellow-brown, with pitted walls about 0.8 μ thick, branched, tapering toward the tips, irregular thickenings along the edge, sinuous, especially in the thinner portions, 3.5–5.5 (8) μ thick; spores globose, concolorous with the capillitium, with a short piece of pedicel attached and pieces of hyaline pedicels mixed with the spores, spiny at a magnification of 440, 4.5–5.5 (6.5) μ in diameter including the spines (mostly 4.5–5 μ in diameter without the spines).

Habitat: on the ground in woods.

Specimens examined in DAOM: 22221, Great Whale River, Que., 24830, Valley Park, Miss., U.S.A.

This species has not as yet been collected from the Ottawa area but has been included here, since it is known to occur in more southerly regions and recently has been collected from Great Whale River in northern Quebec.

The above description has been made mainly from the specimen (DAOM 24830) collected and determined by D. H. Linder and received from the Farlow Herbarium, Harvard University, and from the specimens in the Great Whale River collection (DAOM 22221).

The distinguishing characteristics of *L. echinatum* are the long spines, which at maturity, are dark brown in color and fall off leaving a reticulate inner peridium; the small subgleba with the very small chambers; the spores, which are rough at a magnification of 440; and the pieces of hyaline pedicels mixed with the spores.

Lycoperdon ericetorum Pers. var. ***cepaeforme*** (Bull. ex Pers.) comb. nov.

Lycoperdon cepaeforme Bull. ex Pers., J. de Bot. II: 17. 1809.
Lycoperdon polymorphum Vitt., Mon. Lyc. p. 183. 1843.

Basidiocarp variable in size and shape 9–21 (35) mm broad and 9–25 (45) mm high, globose to subglobose, pear-shaped or with a very short stem-like base and gradually enlarged above into a globose to depressed-globose head, usually plicate beneath, with a stout, cord-like, basal rhizomorph which may be branched and covered with earth, white at first, becoming cream color (R) and finally light brownish olive (R) or brownish olive (R) at maturity; surface may be felty at first, becoming cracked near maturity, but usually composed of granules which become aggregated into warts, or sometimes of spines with their tips convergent, or merely of granules, which remain light-colored and separate from each other revealing the darker, shiny, slightly farinaceous inner peridium; pore apical, 3–5 mm in diameter, very slow to form; gleba white at first, becoming yellowish and finally buffy olive (R) to olive-brown (R) at maturity; subgleba compact, without chambers, white to concolorous with the gleba, occupying up to ¼ of the basidiocarp, usually ⅓ or less; capillitium light to dark yellow-brown, walls up to 1 μ thick, with inconspicuous pits, occasionally septate, frequently branched, tapering towards the tips, sinuous in the thinner portions, irregular thickenings along the edge 3.5–5 (6.5) μ in diameter; spores concolorous with the capillitium, globose, with a small pedicel, smooth or faintly dotted at a magnification of 440, usually minutely spiny at a magnification of 950, 3.5–4.5 μ in diameter.

Habitat: Gregarious to scattered. On the ground in woods and in open places. Aug. to Oct.

Specimens examined in DAOM: 29467, 45100, 45104, 45113, Bell's Corners, Ont.; 22141, 22143, 28017, Lake Opinicon, Ont.; 27415, 27417, 27959, 27993, 45108, North Gower, Ont.; 22204, 27987, 28005, Arboretum, C.E.F., Ottawa, Ont.; 27399, 27422, Ramsayville, Ont.; 28009, Sand Bay, Que.

This is a very variable species in shape and size but can be recognized by

the small, compact subgleba. Most of the specimens collected were small and globose or depressed globose. According to Lloyd (1905*b*) the common form occurring in Europe has a quite large subgleba, and this is considered to be typical for the species, whereas the common form collected here has a small subgleba and is recognized as a distinct variety on this basis.

The form with the small subgleba has been known as *L. cepaeforme* Bull., *L. polymorphum* Vitt., or *L. polymorphum* Vitt. var. *cepaeforme* Lloyd. Perdeck (1950) considered both *L. cepaeforme* Bull. and *L. polymorphum* Vitt. to be synonyms of *L. ericetorum*. He stated that "Persoon's description, plate, and herbarium specimens show that he meant with his *L. ericetorum* only the sessile, subglobose specimens. Vittadini (1842) has already proved that pear-shaped, somewhat stemmed specimens belong to this species". Persoon left three collections of *L. ericetorum* of which HLB 910–258.502 has been designated as the lectotype. Dr. J. W. Groves made notes on this specimen in 1950 and the following information was obtained. The lectotype collection consisted of three fruit bodies and some sections in packets and the fruit bodies were "globose, 1.5–2 cm. in diameter, reddish brown, slightly furfuraceous to almost glabrous", and the spore mass was brown. No mention was made of the characteristics of the subgleba but Perdeck described the subgleba of this species as compact and cited this same specimen. In a mount made from this specimen the capillitium was found to be 2.5–5.0 μ in diameter, lightly colored and branched while the spores were found to be 3.5–5.0 μ in diameter, globose, smooth at a magnification of 440 but minutely roughened at a magnification of 950. This agrees very well with Perdeck's description and with material in our collections.

L. cepaeforme Bull. was treated as a synonym of *L. pratense* Pers. by Persoon (1801) and again as a synonym of *L. ericetorum* Pers. by Persoon (1809). The description of *L. pratense* by Persoon (1801) is inadequate and according to Perdeck (1950) "Persoon left no specimens under this name (except some with a question mark)", therefore this name can be disregarded. Lloyd (1905*a*) has used the name *L. pratense* for a fungus from Australia with hyaline or faintly colored, septate capillitium, and a cellular subgleba separated from the fertile portion by a distinct diaphragm. This species of Lloyd seems to be *L. depressum* Bon. as described by Perdeck (1950) and *L. hiemale* Bull. as described by Cunningham (1942). Lloyd refers to the name *L. pratense* in the following way: "We adopt the name *Lycoperdon pratense*, there being a tradition in Europe that it is Persoon's species and the plant being generally so known now. Still there is no direct evidence on the point. Persoon left no specimens and his figure, which is quite characteristic as to shape, has the surface broken into areas by cracks, never a feature of any specimen I have ever seen. The first definite evidence is that it is *Lycoperdon hyemale* of Vittadini. Not only his specimens exist but he clearly characterizes it, pointing out the peculiar diaphragm so marked in this species". It is evident from the above that there is no legitimate reason for using the name *L. pratense*, and also that Lloyd applied it to a fungus quite different from *L. ericetorum*.

Since our material seems to agree with *L. ericetorum* Pers. except for the smaller subgleba and to agree with Bulliard's *L. cepaeforme* in this respect, I

propose to call our fungus *L. ericetorum* Pers. var. *cepaeforme* (Bull. ex Pers.) comb. nov. to differentiate the form with the small sterile base from that with the large sterile base.

Lycoperdon flavotinctum sp. nov. (Fig. 4).

Basidiocarpus 1–3 cm alt., 1.3–3.5 cm lat., a globoso turbinatus, in primis albus, deinde isabellinus, vel dense luteo-olivaceus, vel luteo-citreus; cortex variabilis, tentus nunc ex spinis pallide brunneis, gracillimis, convergentibus, nunc ex spinis et granulis, nunc ex granulis spinosis; peridium interius pallidum, lucidum; gleba olivaceo-brunnea, cum parva pseudocolumella; subgleba loculosa, ad tertias vel dimidias basidiocarpi lata; capillitium brunneo-luteum, lacunosum, (2.5) 3.5–5 (6) μ diam.; sporae globosae, 4–4.5 (5) μ diam., minute pedicellata, minute spinosa sub 950×.

Habitat: supra solum in locis apertis. Aug.–Oct.

Basidiocarp 1–3 cm high and 1.3–3.5 cm broad, usually broader than tall, variable in shape, usually globose to subglobose or depressed globose, but some obovoid to turbinate, with a short tapering base, slightly plicate beneath, attached to the substratum by a white fibrous mycelium which may form a small pad with the soil at the point of attachment; white at first but becoming a deep isabella color (R) to a dark olive buff (R) or buffy citrine (R); cortex variable, usually composed of pale brownish, minute, slender spines convergent at their tips, or of separate spines and granules, or spinulose granules; in a mixture of both, the spines may disappear almost entirely leaving only the granules; outer coating gradually wearing away and revealing the paler, shiny, inner peridium in places; pore apical; gleba olive-brown, with a small pseudocolumella; subgleba usually concolorous but may be paler to almost white, occupying one-third to one-half of the fruit body, distinctly chambered, convex above; capillitium brownish yellow with distinct darker walls up to 0.8 μ thick, pitted with numerous small pits, branched, a few irregular thickenings along the edge, sinuous, tapering to a blunt point, (2.5) 3.5–5 (6) μ in diameter; spores almost concolorous with the capillitium, globose, with a minute pedicel, minutely spiny at 950, 4–4.5 (5) μ in diameter.

Habitat: on the ground in open places. Aug. to Oct.

Type: DAOM 28004, on sandy ground, Bell's Corners, Ont. Sept. 26, 1951.

Specimens examined in DOAM: 22177, 22198, 27967, 35791, 45091, 45119, 45125, Bell's Corners, Ont.; 22222, Carp, Ont.; 25789, Constance Bay, Ont.; 22159, 22171, 22173, 22179, 22193, 22195, 22196, 22214, 27446, Fitzroy Harbour, Ont.; 22154, 28008, 29476, Merivale, Ont.; 45123, North Gower, Ont.; 27438, Ramsayville, Ont.; 27440, Devereux Lake, Michigan, U.S.A.

This is a relatively common species in this area and is recognized by the yellowish tinge to the fruit body, and by the variable nature of the cortex, which may be composed of either pale brownish, minute, slender spines which may be convergent or single, or of granules or spinulose granules, or a mixture of both. Microscopically the capillitium is characterized by the presence of numerous small pits, while the spores are minutely spiny at a magnification of 950, but appear smooth at a magnification of 450.

L. flavotinctum most commonly has been known throughout the literature as either *L. umbrinum* or *L. turneri*. However, the true *L. umbrinum* Pers. has dark-colored, thin, separate spines which reveal the inner peridium from the beginning, whereas this species has the light brownish, small spines which almost entirely cover the surface from the first.

Our collections seemed to be close to Lloyd's and Morgan's interpretation of *L. turneri* and corresponded well with specimens of *L. turneri* in the Lloyd Herbarium. Lloyd (1905*b*) states that his description of this plant is based "on specimens from J. B. Ellis (given us by Prof. Morgan), and which were (as to the spores at least) the plants originally described. . . . It is the original of Morgan's and in the main of Ellis' description." However, on examining the type collection of *L. turneri* sent to Everhart by L. M. Turner from Labrador in 1884, the collection, which consisted of one fruiting body and part of another, was found to be a *Calvatia* species. The capillitium was broken up into short pieces and ended abruptly with peculiar hyaline projections and the spores were 5–6.5 μ in diameter, minutely roughened, and globose. This, therefore, is obviously not the species meant by either Lloyd or Morgan.

Lycoperdon floccosum Lloyd, Myc. Notes 20: 226. 1905.

Lycoperdon umbrinum Pers. var. floccosum Lloyd, *in* Smith. Puffballs and their allies in Michigan. p. 64. 1951.

Basidiocarp variable in size, 2.5–4.5 cm high, 2–4 cm broad, sometimes broader than tall, pyriform to turbinate, plicate at the base and below the enlarged upper portion, attached by a stout mycelial cord, cream buff (R); cortex a flocculent covering composed of stout, cream-colored (R), floccose warts or spines which either wear off readily or are easily rubbed off leaving a farinaceous to flocculent inner peridium; surface cracking irregularly in these specimens; gleba white at first, becoming yellowish and finally buffy brown (R) with a purplish tint, with a small pseudocolumella; subgleba white at first but becoming olive-brown (R), large chambers, occupying the basal tapering portion or up to about one-half of the basidiocarp, concave above; capillitium a deep yellowish-brown color with distinct walls up to 0.8 μ thick and with a few small pits in the walls, branched, tapering, 4–7.5 (9) μ in diameter in the main threads; spores concolorous with the capillitium, globose, rough at a magnification of 440 and distinctly spiny at a magnification of 950, spines up to 0.8 μ long and projecting into a hyaline envelope which surrounds the spore, 5.5–6 μ in diameter including the spines, 3.5–4.5 μ in diameter without the spines, pieces of hyaline pedicels mixed with the spores.

Habitat: on the ground. Sept.

Specimen examined in DAOM: 25779, 25799, 27971, Lake Opinicon, Ont.

This species is most readily recognized by the flocculent nature of the cortex and inner peridium and by the spores, which are distinctly rough at a magnification of 440.

L. floccosum was first described by Lloyd (1905*b*), who indicated that he considered it to be closely related to *L. umbrinum*. According to Lloyd it is "a form with same internal characters and the same coat of small furfuraceous

spines as *Lycoperdon umbrinum*, but which has a few large, soft, white flocculent spines superficially over the inner coat. It seems to me closest to *umbrinum* but it is analogous to *velatum* if the veil were reduced to a few, scattered, soft, flocculent spines." However, Lloyd's *L. umbrinum* has spores like *L. molle* (his *L. atropurpureum*) and a gleba with a purplish cast at maturity. In one form there is a furfuraceous granular exoperidium and in another form the exoperidium is composed of small spines more regularly fasciculate and persistent. This description of *L. umbrinum* does not agree with the *L. umbrinum* referred to in this paper, but to *L. molle*. Lloyd (1905*b*) himself states that "Many specimens occur that are difficult to refer to either species [*L. umbrinum* or *L. atropurpureum*]." His two species seem to run into each other with only peridial characters to distinguish them.

Our specimens correspond well microscopically with those of Lloyd but on our specimens there seem to be more floccose spines or warts, especially near the base, than on the plant illustrated in Plate 59 (Lloyd 1905*b*).

Smith (1951) treats this same species as *Lycoperdon umbrinum* var. *floccosum* and our specimens correspond well with his illustrations.

Lycoperdon glabellum Peck, N.Y. State Mus. Rep. 31: 39. 1879.

Basidiocarp 2–3 cm high and 1.5–2.5 cm broad, obovoid to turbinate with a short tapering base and a mass of white fibrous mycelium attaching it to the ground, cartridge buff (R) to cream buff (R) at first, but becoming drab (R) to deep olive-brown (R) to buffy brown (R) above and slightly paler below; cortex persistent, furfuraceous, composed of small granules which, when magnified, appear as small floccose warts or spines over the upper surface; inner peridium usually revealed only at the base or in small patches, shiny and almost smooth; pore apical, like a slit, up to 7 mm long; gleba with a small columella, olive-brown (R) to natal brown (R) at maturity (with a purplish tinge); subgleba occupying up to one-half of the basidiocarp, flat to concave above, chambered, paler than the gleba and without the purple color at maturity; capillitium deep reddish brown with distinct walls up to 0.8 μ thick (rarely 1.6 μ) containing a very few small pits, frequently branched, with a few septa, especially near the point of branching, sinuous in the thinner portions, with irregular thickenings along the edge, gradually tapering, (2.5) 3.5–5 (6.5) μ in diameter; spores almost concolorous with the capillitium, globose with a small pedicel, spiny at a magnification of 440 with spines up to 1 μ long at maturity and projecting into and beyond a hyaline envelope which surrounds the spore, 5.5–6 (7) μ in diameter, including the spines, pieces of hyaline pedicels mixed with the spores.

Habitat: on the ground, Sept.

Specimens examined in DAOM: 27983, North Gower, Ont.; 45073, Fallowfield, Ont.; 49650, Fitzroy Harbour, Ont.

L. glabellum is not common in the Ottawa area, but is characterized by the granular to furfuraceous nature of the peridium, by the olive-brown gleba with a purplish tinge, and by the distinctly spiny spores.

Lycoperdon molle Pers., Syn. Meth. Fung. p. 150. 1801. (Fig. 5).

Lycoperdon atropurpureum Vitt., Mon. Lyc. p. 42. 1842.
Lycoperdon umbrinum sensu Hollos, Gastr. Ung., p. 96. 1904.

Basidiocarp 2–5 (6) cm high and 1.5–4 (5.5) cm broad, obovoid to pyriform or turbinate to broadly turbinate or subglobose with a small tapering base, plicate beneath, attached by white fibrils, color variable, from cartridge buff (R) to avellaneous (R) to wood brown (R) to Saccardo's umber (R), usually paler below; cortex composed of light brownish to white, slender spines up to 1 mm long, usually in groups with tips convergent, or of smaller spines and granules mixed with the larger spines in varying proportions, fairly persistent; inner peridium where revealed usually paler in color, smooth to slightly farinaceous, somewhat shiny in places; pore central, apical, 4–6 mm in diameter; gleba white at first but becoming olive-brown and finally dark brown with a purplish tint at maturity, with a small pseudocolumella; subgleba white at first but becoming pale brown or concolorous with the gleba, not sharply separated from the gleba, usually convex above and extending up the sides, occupying ⅓–½ (⅔) of the basidiocarp, cellular; capillitium reddish to yellowish brown with a distinct pitted wall up to 0.8 μ thick, branched, with a few septa, irregular thickenings along the edge, tapering, sinuous in the thinner portions, 3.5–6.5 (7.5) μ in diameter; spores almost concolorous with the capillitium, globose with a minute pedicel, pieces of hyaline pedicels mixed with the spores, rough at a magnification of 440, but distinctly spiny at a magnification of 950 with spines up to 0.8 μ long projecting into a hyaline envelope, (4) 5–6 (6.5) μ in diameter including the spines.

Habitat: on the ground in woods or in sandy places, solitary or in small groups. Aug. to Oct.

Specimens examined in DAOM: 22175, 37585, Bell's Corners, Ont.; 48149, Carp, Ont.; 45093, 45096, 45116, Fallowfield, Ont.; 22147, 48148, Fitzroy Harbour, Ont.; 29485, Kinburn, Ont.; 25784, 25795, 25804, 26258, 26397, Lake Opinicon, Ont.; 22153, 39415, 39418, 39435, Merivale, Ont.; 27430, 27434, 27435, 28022, 45081, North Gower, Ont.; 11210, Forest Belt, C.E.F., Ottawa, Ont.; 22237, 22240, Petawawa Forest Experiment Station, Ont.; 34329, Victoria Beach, Man.; 25780, 26400, Sand Bay, Que.; 21235, Cross Village, Mich., U.S.A.

This is a fairly common but variable species of *Lycoperdon*. The cortex is the most variable character and new species have been based on this great variability, but the different forms all seem to intergrade one into another and for the present have been considered as one. It is easily separated from *L. umbrinum* Pers. (sensu Perdeck) by the larger and distinctly rough spores, with the spines or warts visible at a magnification of 440, and by the pale-colored spines of the cortex, which may be convergent, and may be intermixed with granules.

This species has been called *Lycoperdon atropurpureum* Vitt. by most European and American mycologists. However, according to Perdeck (1950),

who has recently studied Persoon's specimens, *L. molle* Pers. is the proper name for the fungus and *L. atropurpureum* Vitt. is a synonym. From Perdeck's description and from examining a slide made from one of Persoon's specimens marked type (HLB 910.258–472), it is evident that *L. molle* Pers. is the same as the present concept of *L. atropurpureum* Vitt. Lloyd (1907) realized that the two names were synonymous, but did not use Persoon's name probably because, as he stated earlier, he thought that Vittadini's name was more appropriate and that Vittadini was the first to give a clear description of the species.

Hollos (1904) described what appears to be this fungus under the name *L. umbrinum*. He divided this species into a number of varieties based on the peridial characteristics, but his *L. umbrinum* possesses a dark olive to umber or purple-brown gleba, and "strongly warty" spores 5–6 μ in diameter. These characters are typical of *L. molle* and *L. umbrinum* sensu Hollos is, therefore, considered to be a synonym of *L. molle* Pers.

Lycoperdon muscorum Morgan, Jour. Cinn. Soc. Nat. Hist. 13: 16. 1891.

Basidiocarp (1.2) 1.7–3 cm high and 1.4–1.8 cm broad, broadly turbinate or subglobose above, with either a stalk-like or a very short to almost absent, tapering base, plicate beneath and with a little fibrous white mycelium, chamois (R) to deep olive buff (R) above, paler below, cream buff (R) to cream (R) to cartridge buff (R); cortex usually wrinkled, variable, composed either of granules with a few spines or of small simple spines, sometimes with the tips covergent, mixed with floccose granules; inner peridium, where revealed, shiny and paler, but granules quite persistent; pore apical; gleba white at first, but becoming olive-brown at maturity; distinctly separated from the subgleba; subgleba chambered, white at first, but at maturity becoming concolorous with the gleba but often paler at the base, occupying ⅓ to ½ of the basidiocarp or the stalk-like or tapering portion and extending slightly up the sides; capillitium yellow-brown with a distinct, darker, minutely pitted wall up to 0.8 μ thick, branched, occasionally septate at point of branching and often broken up into short pieces, tapering, sinuous in the thinner portions, irregular swellings along the edge, (2.5) 3.5–5 (6.5) μ in diameter; spores concolorous with the capillitium, globose with a short piece of pedicel attached, smooth but some slightly roughened at a magnification of 440, minutely spiny at a magnification of 950, 4–4.5 (5) μ in diameter (without the spines).

Habitat: among moss or on shady ground. Sept. to Oct.

Specimens examined in DAOM: 27978, Bell's Corners, Ont.; 27974, Merivale, Ont.; 28012, Merivale Road, Ont.; 25786, Sand Bay, Que.; 24737, Riverdale, Mich., U.S.A.

L. muscorum is not often found in the Ottawa area. It is characterized by the presence of a stalk or very short tapering base, and by the capillitium, which breaks up into short pieces much as it does in the genus *Calvatia*, although the fruit body opens by a pore. The specimens collected have been fairly small, but Lohman (1927) has reported that the fruit bodies may reach a height of 6 cm and a diameter of 3 cm.

According to Morgan (1891) "This is *L. molle* of Peck's U.S. species of *Lycoperdon*."

Lycoperdon pedicellatum Peck, N.Y. State Mus. Rep. 26: 73. 1874. (Fig. 10).

Lycoperdon caudatum Schroeter, Pilze Schles. 3: 698. 1889.
Bovistella pedicellata Lloyd, Myc. Writings 2: 282. 1906.

Basidiocarp 1.5–3.5 cm broad, 1.7–4 cm. high, obovoid to turbinate or with a thick short stipe and a globose to subglobose head and plicate below, attached to the substratum by white rhizomorphs, white at first but becoming cream (R) to old gold (R) or isabella color (R) above, and cream color (R) to chamois (R) below; outer peridium composed of stout dehiscent spines, up to 1 mm thick at the base and 1–2 mm long, with the tips often convergent and with the bases fairly close together, the upper spines falling off leaving a smooth or obscurely reticulated or shallow pitted pale olive-buff (R) to greyish olive-buff (R) inner peridium; the lower spines smaller, finer, usually farther apart, occasionally with granular material mixed in, often darker and quite persistent; gleba white at first, but becoming cream and finally light brownish olive (R) to olive-buff at maturity; subgleba white but becoming cream to almost concolorous with the gleba or with a purplish tint, of small to medium-sized chambers, occupying up to one-half of the basidiocarp in stalked forms but very small in others, convex above, extending up the sides; capillitium highly colored, deep yellowish to reddish brown, with distinct darker walls up to 1 μ thick, distinctly pitted (few to fairly numerous pits), frequently branched, sometimes septate (some threads broken up into short pieces which indicate a few septa), gradually tapered towards the ends, with some irregular thickenings along the edge, sinuous in the thinner portions, 3.3–5.7 (9.8) μ in diameter; spores lighter than the capillitium, globose to subglobose to slightly oval, smooth to slightly roughened with the spines projecting into a thin, hyaline envelope which surrounds the spores, 3.5–5 $\times$ 3.5–4 μ with hyaline pedicels up to 30 (40) μ long.

Habitat: on ground and on rotted wood. Aug.–Oct. (Nov.).

Specimens examined in DAOM: 29488, Cantley, Que.; 26234, Dunham, Que.; 29449, 45107, Fallowfield, Ont.; 27406, 27448, 27982, Gatineau Park, Que.; 25802, 25809, Lake Opinicon, Ont.; 39416, Merivale, Ont.; 21312, Ottawa, Ont.

This puffball is easily recognized by the rather long, deciduous, whitish spines and by the characteristic spores with the long hyaline pedicels. It seems to be a rare plant in the Ottawa district although according to American mycologists it is more frequently found in northern areas.

Lloyd (1906) placed this species in *Bovistella* as he includes in this genus those species with the *Bovista* type of capillitium and those whose spores have permanent pedicels or either one of these characteristics, and states that they could be called either a *Bovistella* or a *Lycoperdon*. However, most authors have treated the genus *Bovistella* as possessing both these characteristics, thus excluding *L. pedicellatum*.

Lycoperdon perlatum Pers., Syn. Fung. p. 145. 1801.

Lycoperdon gemmatum Batsch ex Fr., Syst. Myc. 3: 36. 1829.

Basidiocarp variable in size (1.2) 2–4 (5) cm high by (1) 2–3 (5) cm broad, variously shaped, turbinate to obovoid, to almost pyriform, or subglobose above with a stem-like base, which may be plicate at both the base and the top, and may have a swollen base; usually white when young but becoming isabella color (R) to buffy brown (R) to Saccardo's umber above but usually remaining cream buff (R) to warm buff (R) below at maturity; cortex composed of white to brownish, simple, stout spines or warts, or a number of thinner ones closely joined together, or of long, dark-colored or pale, slender, curved spines in groups of two to six, with small warts or granules which may surround the large warts or groups of spines in either a regular or irregular manner; spines and warts also extend down the stalk where they are much smaller and the difference in size is less pronounced; spines and warts fall off at maturity, leaving either a smooth surface or pale areolae which give the inner peridium a reticulate appearance which may disappear with age; pore apical, large, up to 5 mm in diameter; gleba white at first, turning olive-brown at maturity, sometimes with a purplish tint, and with a distinct pseudocolumella; subgleba coarse, variable in color from white to almost concolorous with the gleba, occupying the stalk-like portion and extending up the sides; capillitium light to medium yellow-brown with a distinct wall up to 0.8 μ thick, not or only slightly pitted, branched, irregular thickenings along the edges, sinuous in the thinner portions, tapering, (1.5) 2.5–5 (7.5) μ in diameter, some hyaline threads present; spores almost concolorous with the capillitium, globose, some with a small piece of pedicel still attached, minutely spiny at a magnification of 950, 3.5–4 (5) μ in diameter.

Habitat: Single, gregarious, or caespitose. On rotten logs and stumps and more usually on the ground. Aug.–Oct.

This is one of our most common species, but it is very variable in shape, size, and color, and has often been referred to as the gem-studded puffball as the erect warts or spines often give the surface the appearance of being studded with gems. The short warts are more abundant on the upper surface and as the plants mature, these warts fall off leaving pale areolae which usually give the peridium a reticulate appearance.

In some collections the specimens are quite a different shape from usual. The fruit bodies consist of a long, nearly cylindrical stalk which may be slightly swollen at the base and with a subglobose head. Typical *L. perlatum* spines cover the head and smaller ones extend down the stalk. There are intergradations between this form and the typical form and Lloyd (1905*b*) illustrated several forms in Plate 46.

For many years *Lycoperdon gemmatum* Batsch ex Fr. and *Lycoperdon perlatum* Pers. were thought to be separate species. According to Massee (1887), *L. perlatum* was characterized by the presence of a columella and an umbo, and *L. gemmatum* by the absence of these characters. Morgan (1891) also distinguished the two on the color of their spines, *L. gemmatum* with white or grey spines and *L. perlatum* with greyish brown or black spines. However,

Lloyd (1905*b*) states that he "never could grasp the difference between them" and that "Persoon's specimens of *perlatum* are the ordinary form of *gemmatum* tout-a-fait." In 1801 Persoon used *L. gemmatum* as a synonym of *L. perlatum*, and Hollos (1904), Kambly and Lee (1936), and Perdeck (1950) consider the two species to be synonymous. Since *L. perlatum* Pers. and *L. gemmatum* Batsch. ex Fr. are the same species and since *L. perlatum* was described in 1801, the starting point of nomenclature in the Gasteromycetes, *L. perlatum* Pers. is to be considered as the correct name for this fungus.

Several varieties of *L. perlatum* have been described. Perdeck (1950) included three varieties based on the structure of the exoperidium. *L. perlatum* Pers. var. *typicum* and *L. perlatum* Pers. var. *bonordenii* (Mass.) Perdeck both have big spines which are either simple or thick, or consist of a number of thinner ones. In var. *typicum* after falling off they leave a very distinct reticulate structure on the cortex, the big spines being surrounded by smaller ones and leaving paler areolae, whereas in var. *bonordenii* there is no distinct reticulate structure, either because there is no distinct differentiation between big and small spines, or else the small spines are very rudimentary, or do not surround the big ones regularly. In the third variety, var. *nigrescens* Pers., the big spines are dark-colored, slender and curved, standing together in little groups (usually four to six) and at most grown together only at the base and top of the spines and mostly with a distinct reticulate structure on the cortex. Lloyd (1905*b*) considers *L. nigrescens* to be a separate species, but states that it "is really a form of *gemmatum* excepting that the stiff spines are not consolidated." He included *L. excoriatum* as a form of *L. perlatum*. This form is characterized by the warts adhering together and flaking off in pieces and leaving a smooth endoperidium.

The following forms have been recognized in this area and have been separated mainly on the structure of the exo- and endo-peridium.

Key to Varieties

1. Inner peridium reticulate at maturity 2
1. Inner peridium not reticulate at maturity 4
2. Cortex of simple thick spines or warts, or of a number of thinner ones closely joined together ..... var. *perlatum*
2. Cortex of slender, curved spines in little groups 3
3. Spines brownish to white ..... var. *peckii*
3. Spines dark-colored ..... var. *nigrescens*
4. Spines and warts falling off singly ..... var. *bonordenii*
4. Spines and warts flaking off ..... var. *excoriatum*

Lycoperdon perlatum Pers. var. *perlatum*, Blumea, 6: 505. 1950.

Cortex composed of large, simple, thick, white or brownish spines or warts, or of a number of thinner, white or brownish spines closely joined together. The large spines are surrounded by small warts and granules, which may fall off leaving distinct pale areolae, and a reticulate surface.

This is the most common variety and it is only occasionally that one of the other varieties is found. The specimens range in color from almost white to a dark brown, and the character of the exoperidium is equally variable. In some cases the white warts have black tips, while in others the warts are concolorous

varying from white through brownish to very dark brown. The size of the warts is very variable and a hand lens is often required to pick out the characteristics of the exoperidium. This species is truly a very variable one and it is not until many specimens are examined that the intergradations of the exoperidial structure can be clearly understood.

Specimens examined in DAOM: 22149, 22186, 22197, 27408, 27997, 45101, Bell's Corners, Ont.; 22228, 22233, Carp, Ont.; 11186, Dunrobin, Ont.; 22199, 22223, Fitzroy Harbour, Ont.; 25792, 25793, Lake Opinicon, Ont.; 22148, 22156, 26232, 26237, 26239, 26259, 27427, Merivale, Ont.; 27412, 27421, 27431, 27441, 27985, 28023, 28024, 45120, North Gower, Ont.; 5720, 7796, 16775, 22238, 22241, Petawawa Forest Experiment Station, Ont., 28015, Ramsayville, Ont.; 11181, 11187, Burnet, Que.; 34330, Danford Lake, Que.; 26236, Dunham, Que.; 16774, 16781, 16788, Eardley, Que.; 11193, Gatineau Park, Que.; 25782, 25790, Kingsmere, Que.; F9664, Ste. Anne de la Pocatière, Que.; 34328, St. Aubert, Que.; 25791, Sand Bay, Que.; 27965, Taylor Lake, Que.; 39395, 39441, Kentville, N.S.; 39414, Moore's Falls, N.S.; 39410, Glenmont, Kings Co., N.S.; 39430, Cape Split, Kings Co., N.S.; 27404, Vancouver, B.C.; 29468, Ithaca, N.Y.; 27995, Lower Tahquamenon Falls, Michigan.

Lycoperdon perlatum Pers. var. *bonordenii* (Mass.) Perdeck, Blumea, 6: 505. 1950.

Lycoperdon bonordenii Mass., J. Roy. Micr. Soc. p. 713. 1887.

Cortex composed of coarse warts which are simple and thick and close together with only a few small warts and granules irregularly arranged between the large warts, which reveal no distinct reticulate structure on the endoperidium when the large warts fall off.

Specimens examined in DAOM: F9529, Ingolf, Ont.; 22224, Merivale, Ont.; 27420, North Gower, Ont.; 22205, Kingsmere, Que.

Lycoperdon perlatum Pers. var. ***excoriatum*** (Lloyd) comb. nov.

Lycoperdon excoriatum Lloyd, Myc. Writ. 2: 229. 1905.

Basidiocarp with a fairly cylindrical stalk which changes gradually into a subglobose head; cortex composed of large or smaller spines or warts (of the typical var. *perlatum* type) surrounded by smaller spines or warts and granules, usually flaking off, leaving a smooth, shiny, light olive-tan inner peridium.

Specimens examined in DAOM: 27977, 28011, North Gower, Ont.; 17163, Granby, Que.

Lycoperdon perlatum Pers. var. *nigrescens* Pers., Syn. Meth. Fung. p. 146. 1801.

Cortex composed of large, slender, curved, dark-colored spines which are surrounded by smaller spines and warts. Large spines up to 2 mm long and standing together in clusters of three to six, sometimes with their bases united and convergent at the apex, and on falling off reveal pale areolae which give the inner peridium a reticulate surface. Pieces of hyaline pedicels may be mixed with the spores.

Specimens examined in DAOM: F9538, Ingolf, Ont.; 28003, Petawawa Forest Experiment Station, Ont.

Lycoperdon perlatum var. ***peckii*** (Morg.) comb. nov.

Lycoperdon peckii Morgan, J. Cinn. Soc. Nat. Hist. 13: 15. 1891.

Cortex composed of tapering whitish to pale brownish spines up to 1.5 mm long, which usually split only at the base into two to four parts, and are convergent at the apex in little stellate groups, with small warts or nodules regularly arranged between the spines so that when the large spines fall off a reticulate inner peridium is revealed.

Habitat: on the ground. Sept.

Specimens examined in DAOM: 27989, 29452, Bell's Corners, Ont.; 26299, Merivale, Ont.

This variety appears to be rare in this area, but is easily distinguished from var. *nigrescens* by the pale color of the spines and from the other varieties by the fascicled spines with flaring bases and cohering tips.

Our specimens are immature but closely resemble the description and illustrations of *L. peckii* as given by Coker and Couch (1928), except the large spines are described as having a "delicate and pretty purplish lavender color" when fresh and as changing to a buffy-brown at maturity. The lavender color cannot be seen in the dried specimens and since our plants are still immature, the color of the spines is very pale.

Morgan (1891) stated that his *L. peckii* is *L. echinatum* sensu Peck (1879), and Coker and Couch (1928) examined plants in the Ellis collection at the New York Botanical Garden "from Peck labelled *L. echinatum* which are *L. peckii*." They found that their plants agreed with those of Peck's, although Peck (1879) described the plants as having a smooth inner peridium. According to Coker and Couch (1928), "A difference in age of plants might easily account for such a discrepancy."

Coker and Couch (1928) stated that the "most important difference between the present species [*L. peckii*] and *L. gemmatum*, which is nearest, and of which this might be considered as a variety, is the tapering fascicled spines with tips united." Coker and Couch's (1928) concept of *L. peckii* has been followed, but the species has been considered as a variety of *L. perlatum*.

Lycoperdon pulcherrimum B. & C., Grevillea, 2: 51. 1873.

Lycoperdon frostii Peck, Trans. Albany Inst. 9: 301. 1879.

The following description was copied from Coker and Couch, p. 72–73.

"Plant 1.8–4.5 cm. broad and about the same height, tapering below to a slender root, covered at first with very long, slender, pure white then pale tan spines, which are united into groups by their tips and which finally fall away from the upper part of the plant, leaving the smooth, shining, deep brown, purplish brown or silvery brown surface of the inner peridium exposed and not reticulated. Gleba white then passing through olivaceous to dark purple-brown; subgleba occupying about one-third or slightly more of the

plant. Odor when maturing distinctly aromatic, somewhat like that of *L. marginatum*, but not nearly so offensive.

Spores (of No. 477) with a large oil drop and no obvious stalk, globose, set with short blunt spines, 4–4.7 μ thick, not counting the spines, average about 5 μ with spines. Capillitium much branched, long and tapering, the lesser branches about the size of the spores, the main branches up to 8.6 μ in diameter in places."

A collection (DAOM 27445) of an immature fungus which seemed to be *L. pulcherrimum* was made near Lake Opinicon, Ontario, at the Queen's Biological Station and for this reason a description of the species has been included. The Lake Opinicon specimens had long white spines which seemed to correspond well with those illustrated by Morgan (1891), but none of the internal characteristics of the fungus could be determined due to the immaturity of the fruit bodies.

L. pulcherrimum is characterized by its long white spines which leave a smooth inner peridium when they fall away, and by the spores which are warty at a magnification of 440.

Lycoperdon pusillum Persoon, Jour. de Bot. 2: 17. 1809. (Fig. 8)

Basidiocarp 7–19 mm in diameter, globose to subglobose, sessile, sometimes plicate beneath, with a slender or stout, cord-like, basal rhizomorph or with a few branched, white rhizomorphs attaching it to the substratum; white when immature, becoming buff and finally a light brownish olive (R) to a dull brown at maturity; cortex may be felty to floccose at first, usually becoming aggregated into a dense covering of granules or of spines convergent at their tips and separating from each other on drying, or breaking up into areolate patches and shrinking to flake-like particles which are slowly deciduous, or the surface may be mealy or furfuraceous, often resembling dried up soap suds; inner peridium paler, somewhat farinaceous, becoming shiny; pore apical, slow to form, often lobed, 2–5 mm in diameter; gleba white at first, becoming pale yellow, olive-brown, and sometimes a deep brown; subgleba absent; capillitium light to medium brownish yellow, walls distinct, usually up to 0.8 (1.5) μ thick, with inconspicuous pits in the larger threads, branched, occasionally septate, tapering gradually to the tip with tapered portions paler and sinuous, some irregular thickenings along the edge, 3.5–5 (6.5) μ in diameter; spores concolorous with the capillitium, globose, with a minute pedicel, smooth to faintly dotted, at a magnification of 440, smooth to faintly dotted to minutely spiny at a magnification of 950, 4–5 μ in diameter.

Habitat: Densely gregarious, subcaespitose, or scattered on sandy soil, in woods or in pasture. Aug.–Oct.

Specimens examined in DOAM: 27990, 27991, 27992, 28020, 29453, 29455, 29456, 29458, 29460, 45103, 45114, 45949, Bell's Corners, Ont.; 25783, 27400, 27437, Fitzroy Harbour, Ont.; 29495, Franktown, Ont.; 25781, Lake Opinicon, Ont.; 27975, 28018, Merivale, Ont.; 27401, 27413, 27414, 27968, 29495, 45076, 45078, 45086, North Gower, Ont.; 27407, Ottawa, Ont.; 16760, Petawawa Forest Experiment Station, Ont.; 34553, 37592, Kingsmere, Que.; 25785, Sand Bay, Que.; 39421, Moore's Falls, N.S.

Lycoperdon pusillum is one of the common small species of *Lycoperdon*. It is readily distinguished by its small size, by the absence of a subgleba, and by the granular nature of the cortex, which often resembles dried up soap suds. The smaller mature forms of *L. curtisii* B. & C. may be confused with this species but they are readily distinguished from the latter by the presence of a subgleba and hyaline capillitium.

L. pusillum has been included as a synonym of *L. ericetorum* by Perdeck (1950), as he states that "Persoon (1809) distinguishes *L. pusillum* only by its small size." Most authors have treated *L. pusillum* as a separate species, distinguishing it by the absence of a subgleba. However, Perdeck (1950) does not agree with this as he "found all transitions between big and small specimens, even growing in the same spot" and he also "found all stages between the absence and the strong development of the subgleba in specimens in one locality." Perdeck's term 'locality' is rather indefinite and could easily refer to a number of specimens collected in the same area, rather than a single collection, and so the presence or absence of the subgleba does not bear too much significance in this case. Our specimens have all been of the same type from one collection and have been without the subgleba even though a collection of the *cepaeforme* form of *L. ericetorum* has been found close by. For this reason, we prefer to keep *L. pusillum* as a separate species.

Lycoperdon pyriforme Pers., Syn. Meth. Fung. p. 148. 1801. (Fig. 7)

Basidiocarp up to 4.5 cm high and 3 cm broad, ovoid to pyriform, sometimes slightly plicate beneath with abundant white fibrous mycelium, white at first but becoming cinnamon buff (R) and finally tawny olive (R), Saccardo's umber (R) to snuff brown (R); outer coat a thin, persistent coat of minute reddish-brown, furfuraceous scales, granules or spinules, which are rough to the touch (like a file) and may partly disappear exposing the yellow-brown, smooth, inner peridium, or the cortex may be divided into areolae which redivide into smaller indistinct units of small granules; pore apical, slow to form, up to 6 mm in diameter; gleba white at first, becoming light brownish olive (R), with a distinct columella; subgleba white to creamy or pale brown, composed of small cells, occupying the stem-like base or tapered portion and extending up the sides; capillitium dark reddish brown to yellow-brown, with distinct darker walls up to 1.5 μ in some threads, branched, attenuated toward the ends and sinuous and paler in the thinner portions, 3.5–5 (6.5) μ in diameter, often mixed with hyaline septate threads; spores lighter than the capillitium, globose, smooth 3.5–4 (5) μ in diameter.

Habitat: Usually caespitose, sometimes in very large clusters and sometimes single. On rotten logs and stumps or on ground rich in wood debris. Aug. to Oct.

Specimens examined in DAOM: 25777, Algonquin Park, Ont.; 29498, Bell's Corners, Ont.; 4230, Brant Co., Ont.; 39419, Combermere, Ont.; 25800, Dorset, Ont.; 22165, Fitzroy Harbour, Ont.; 29466, Moore Lake, Haliburton Co., Ont.; 27984, North Gower, Ont.; 22207, 29672 (tessellatum form), 5748, Ottawa, Ont.; 16765, 16773, 16267 (desmazières form), Petawawa Forest Experiment Station, Ont.; 25803, Telephone Creek, Queen's Biological

Station, Ont.; 25776, 27462, 37589, Kingsmere, Que.; 45088, Meach Lake, Que.; 16964, St. Aubert, Que.; 39404, Aylesford Lake, N.S.; 39411, 39423, Kentville, N.S., 27438, Ithaca, Mich., U.S.A.

Lycoperdon pyriforme can be easily recognized by its pyriform shape, by the fairly persistent character of the outer peridium which feels like a file when the tip of the finger is run over the surface, and by the smooth, globose spores. Cunningham (1942) has reported that all the spores he has examined are provided with delicate verrucae, but these have not been observed on spores from our collections.

Hollos (1904) distinguished three varieties of *L. pyriforme*: var. *excipuliforme* Desmaz. with an elongated stalk; var. *tessellatum* Pers. with angular and round areas and reticulate surface; and var. *serotinum* (Bon.) Hollos with flat brownish-red plates or little spines. Lloyd (1905*b*) distinguished five varieties or forms and gave them all specific rank although he considered them to be forms of *L. pyriforme*: *L. serotinum*, *L. tessellatum*, *L. desmazieres* (var. *excipuliforme* of Hollos), *L. faveolum* Lloyd with the surface covered with large pits, and *L. pseudoradicans* Lloyd with the sterile base developed into a root like projection.

In the Ottawa area, there are two main forms of *L. pyriforme*, the typical form with the surface appearing smooth and the other with an areolate surface. The areolate surface appears to be very similar to that shown in Lloyd's Plate 50, Figs. 1 and 2, and Plate 61, Figs. 1–3, of *L. serotinum* (Lloyd 1905*b*). One collection of both var. *tessellatum* and var. *excipuliforme* has been found in this area. However, it is difficult to determine whether these forms are true varieties and whether the characteristics are constant as some of them may be due to unusual climatic conditions.

Lycoperdon rimulatum Peck in Trelease, Trans. Wisc. Acad. Sci. Arts and Letters, 7: 117. 1889.

Basidiocarp depressed-globose or flattened, with or without a small umbo above, 1.6–4 cm broad and 1.4–2 cm high, nearly always broader than tall, plicate below with a small slender root-like projection of fibrous mycelium, white at first but becoming cinnamon-buff (R) to tawny olive (R) with a purplish cast from the spores, paler below; outer peridium smooth and felt-like when young, soon becoming rimose, finally rimulose, sometimes indistinctly so, and thinner as the plant matures, gradually flaking off revealing a shiny to slightly farinaceous, wrinkled, deep olive-buff (R) or paler, inner peridium, often with a purplish cast from the spores; pore apical, up to 5 mm in diameter; gleba white at first, changing to yellow, olive-brown, and finally purple-brown at maturity; subgleba becoming concolorous with the gleba, cellular, broad, and flattened, occupying up to one-quarter of the fruit body; capillitium dark yellow-brown to chocolate brown, with distinct darker walls up to about 1 μ thick (occasionally 1.5 μ), simple, occasionally branched and with a very few septa, irregular thickenings along the edge, tapering, smaller than the spores, 3.5–5 (6.5) μ in diameter; spores concolorous with the capillitium or sometimes with a purplish tinge, globose, distinctly warted with the warts embedded in a hyaline envelope which surrounds the spore, 5.5–7.5 (8) μ in diameter

including the warts, pieces of hyaline pedicels up to 25 μ long still attached to some of the spores or lying free in the mount, leaving a short basal part attached to the spore when they break off.

Habitat: on the ground in fields or pastures. Aug.–Oct.

Specimens examined in DAOM: 22151, 22160, 25778, 25807, 29484, Fitzroy Harbour, Ont.; 39391, 39409, Kentville, N.S.

L. rimulatum is an uncommon species in the Ottawa area and appears to be quite variable in the characteristics of the cortex. When very young, the plants are white with a fairly thick, felt-like cortex which quickly becomes rimose over the upper surface. As the plant matures a few nodules may form near the pore, the cortex darkens, becomes more finely divided, much thinner, and finally peels or flakes off leaving the inner peridium, which may become very wrinkled. The degree of rimulation at maturity varies considerably from one plant to another, and if the outer peridium has fallen off, there is no evidence of the rimulose nature of the plant. The plants, when mature, have a purple-brown gleba and a small concolorous, cellular subgleba. The spores are strongly warted and including the warts, are larger than those in other species of *Lycoperdon*, while the capillitium threads are of a smaller diameter than the spores.

Lloyd (1905*b*) considered *L. subvelatum* Lloyd to be a southern form of *L. rimulatum* in which the cortex is thick and breaks into distinct areas which loosen and fall away. From mounts made from the two specimens from Florida in Lloyd's Herbarium (Lloyd 50644 and 50645) the spores and capillitium were found to be the same as in a typical *L. rimulatum*, with the exception that spores 8 μ in diameter occurred more frequently. Microscopically these two species are the same, but the cortex in *L. subvelatum* is thicker and divided into distinct areas in No. 50645 from Lloyd's herbarium. This collection is from Grasmere, Florida, was collected by C. H. Baker, and should be considered the type of *L. subvelatum* as it is the fruit bodies in this collection which Lloyd illustrated in Plate 56, Figs. 10 and 11 (1905*b*) and on which he based his species or form. From the microscopic characteristics, it would seem that *L. subvelatum* is merely a form or variety of *L. rimulatum*. However, before any definite conclusions can be drawn, it is necessary to examine a much larger number of fruit bodies.

Lycoperdon spadiceum Persoon, J. de Bot. 2: 18. 1809.

Basidiocarp 10–17 (25) mm in diameter, globose to subglobose, sessile, slightly plicate beneath and with a small, cord-like, basal rhizomorph which becomes covered with soil; white at first but becoming cream buff (R) chamois (R), isabella color (R), or dark olive-brown (R) at maturity; cortex at first composed of floccose granules which may become aggregated to form small spinulose granules with their tips convergent at first, but on drying the granules separate, gradually falling off to reveal the inner peridium, which is shiny, smooth and somewhat farinaceous; pore apical, slow to form, 2–3 mm in diameter; gleba white at first but becoming yellowish and finally olive-brown at maturity, fairly distinctly separated from the subgleba; subgleba

concolorous with the gleba or sometimes with a purplish tinge, cellular, occupying up to one third of the basidiocarp, often considerably less, capillitium light brownish yellow with a distinct darker wall up to 0.8 μ thick, but usually thinner, conspicuously pitted, branched, tapering toward the tips, occasionally septate at the point of branching, sinuous and paler in the thinner portions, irregular thickenings along the edge, 3.5–5 (6.5) μ in diameter; spores concolorous with the capillitium, globose, mostly with a small pedicel, usually smooth at a magnification of 440, but minutely spiny or faintly dotted at a magnification of 950, (3.5) 4–5 μ in diameter.

Habitat: on the ground, Aug.–Oct.

Specimens examined in DAOM: 29496, Franktown, Ont.; 27403, 27980, Merivale Road, Ont.; 39433, Ottawa, Ont.

Lycoperdon spadiceum Pers. is a new species in the Ottawa district and this may constitute the first report of the species in Canada. Peck (1879) described a common fungus which he called *L. pusillum* Fr. which is like our fungus, whereas MacBride and Allin (1896) described an uncommon fungus like both Peck's and the fungus under discussion and called it *L. pusillum* Batsch. However, since the fungi described as *L. pusillum* by Peck (1879) and MacBride and Allin (1896) have a cellular subgleba they are not the same as *L. pusillum* Pers. or as *L. ericetorum* Pers. var. *cepaeforme* (Bull. ex Pers.) n. comb. and could very well be *L. spadiceum* Pers.

Lycoperdon spadiceum Pers. can easily be distinguished from *L. ericetorum* var. *cepaeforme* with the compact subgleba and *L. pusillum* without a subgleba, by the presence of its small cellular subgleba, although the other characteristics are the same.

The above description was made from our own collections of what seems to be this species and differs somewhat from Perdeck's description. Perdeck (1950) described the fungus as being 1.2–5 (9) cm high and 1–4 (6) cm broad with a "height–width ratio of 2/1 to 3/4 sessile or with a more or less distinct stem." He described the surface as sometimes having white granules, but made no mention of pits in the capillitium. With only the few collections we have at our disposal it would be difficult to find the same amount of variation in the size of the fruit bodies. Otherwise, our fungus seems to be almost identical with that described by Perdeck.

Cunningham (1942) definitely distinguishes *L. spadiceum* by the freely branched capillitium and cellular subgleba, and Lange (1948) has reported this same species from Greenland and both describe it as a small species reaching a diameter of 24 mm. According to Perdeck, *L. molle* of Morgan, Hollos, and others may be this species.

Lycoperdon subincarnatum Peck, Ann. Rep. N.Y. St. Mus. 24: 83–84. 1872. (Fig. 9).

Basidiocarp 1–2.5 cm broad and 1–2.5 cm high, depressed-globose, globose, or short pyriform, usually sessile and some with a broad point of attachment, sometimes plicate beneath, attached by strands of white fibrous mycelium, usually buffy brown (R) to wood brown (R), but also close to olive-brown

(R); cortex at first composed of brown to reddish brown, small, separate, subpyramidal warts or small spines with their tips connivent, which slowly wear away revealing the paler, pinkish buff (R) to avellaneous (R), pitted, inner peridium at maturity; inner peridium firm, tough, marked all over by pits outlined by acute ridges, like a thimble, but pits may be evident through the warts and spines; pore apical, 3–4 mm in diameter, conspicuous; gleba with distinct radiating lines when young, white at first, becoming yellowish and finally tawny olive (R) to Saccardo's umber (R) and often with a greyish tinge; subgleba white to light straw-colored to light brownish, usually very small, but may occupy up to one-third of the basidiocarp; capillitium hyaline or nearly so, septate, rarely branched, walls variable in thickness, usually thin, often encrusted, irregular thickenings along the sides, 3.5–6.5 μ in diameter; spores light yellowish brown, globose, with a minute pedicel, rough at a magnification of 440, small spines at a magnification of 950 with spines projecting into a thin, hyaline envelope which surrounds the spores, 4–5 μ in diameter.

Habitat: usually caespitose, on dead wood. Late Aug.–Oct.

Specimens examined in DAOM: 22166, 27996, 29492, Bell's Corners, Ont.; 39440, Dorset, Ont.; 16752, Dunrobin, Ont.; 5719, 5737, 11901, 16766, Petawawa Forest Experiment Station, Ont.; 7794, Blue Sea Lake, Que.; 7795, Calumet, Que.; 29487, Cantley, Que.; 2516, Chelsea Station, Que.; 22200, Eardley, Que.; 29447, Fortune Lake, Que.; 22176, 27439, 45117, Gatineau Park, Que.; 37586, Kingsmere Road, Que.; 26235, Meach Lake, Que.; 39413, Cape Split, Kings Co., N.S.; 45071, Gainesville, Fla., U.S.A.

L. subincarnatum Pk. is found on decaying wood and may be either caespitose or gregarious. It is easily recognized by its habitat on decaying wood, the pitted inner peridium like a thimble, the pinkish brown color of the cortex and the thin-walled, septate, hyaline or nearly hyaline capillitium.

Lycoperdon umbrinum Pers., Syn. Meth. Fung. p. 147. 1801. (Fig. 6).

Basidiocarp 1.5–4 cm high and 1.5–2.5 cm broad, pyriform with a tapering base to broadly turbinate or subglobose with or without a short base, plicate beneath, attached by white fibrous mycelium, variable in color, cream-buff (R) to chamois (R) to honey-yellow (R) to clay (R), usually paler below; outer peridium mostly persistent, composed of small, dark, slender spines up to 0.5 (1.0) mm long, usually well separated, and smaller spines and a few granules, some larger spines may be convergent but never in regular groups; inner peridium smooth, shiny and paler in color, revealed between the spines and granules or by the falling off of the spines; pore apical, 3–5 mm in diameter; gleba white at first but becoming olive-brown at maturity with a small pseudocolumella; subgleba varying from white to olive-brown to brown with a purplish tinge, occupying one-third to one-half of the basidiocarp, cellular; capillitium yellow-brown with walls up to 0.8 (1.6) μ thick, pitted, branched, occasionally septate, irregular thickenings along the edge, tapering, sinuous in the thinner portions, 3.5–5 (6.5) μ in diameter; spores almost concolorous with the capillitium, globose with a small pedicel, minutely spiny at a magnifi-

cation of 950 with some appearing smooth, 4–5 (5.5) μ in diameter including spines.

Habitat: on the ground, usually in woods. Aug.–Nov.

Specimens examined in DAOM: 29469, 28019, Bell's Corners, Ont.; 22170, Constance Bay, Ont.; 48147, Fallowfield, Ont.; 22184, Fitzroy Harbour, Ont.; 27429, Merivale, Ont.; 27402, 27418, 28021, 45074, 45080, 45094, 45095, North Gower, Ont.; 27444, 28014, Ramsayville, Ont.; 26233, Dunham, Que.; 45098, Speaker's Falls, Gatineau Park, Que.; 27398, 27966, Wolff Bog, Mich., U.S.A.

This species is recognized macroscopically by the presence of the dark-colored, slender spines and the olive-brown gleba, and microscopically by the spores which appear smooth at a magnification of 440 but are minutely spiny at a magnification of 950. The dark, slender spines are variable in size, mostly about 0.5 mm long but some longer with shorter ones and occasionally a few spinulose granules; they usually occur singly rather than in groups and cover nearly the whole surface of the fruit body.

This is not the concept of the species according to many authors who have included in *L. umbrinum* specimens with spores distinctly warty at a magnification of 440 and with pale-colored long or short spines and granules frequently mixed together or specimens with minutely warted spores at a magnification of 950 and pale-colored small spines and granules. Perdeck (1950), who had studied Persoon's specimens, designated a lectotype (HLB 911.81-14) of the species and provided a description. A slide from this lectotype specimen was examined and the spores and capillitium of our specimens were the same as those on the slide. Therefore we feel that the specimens listed above are *Lycoperdon umbrinum* Pers.

Acknowledgments

The author wishes to express her deep appreciation to Dr. J. W. Groves for his assistance during this study and the preparation of the manuscript. Type specimens have been loaned by Dr. D. P. Rogers, New York Botanic Gardens, and Dr. S. J. Smith, New York State Museum; Mr. J. A. Stevenson at the U.S. National Fungus collections, Beltsville, Maryland, allowed me to examine Lloyd's material, Mr. R. A. Maas Geesteranus supplied information about the capillitium and spores of *L. candidum* Pers., and Dr. B. Boivin supplied the latin description for the new species.

References

COKER, W. C. and COUCH, J. N. 1928. The Gasteromycetes of the Eastern United States and Canada. University of North Carolina Press, Chapel Hill, N.C.

CUNNINGHAM, G. H. 1942. The Gasteromycetes of Australia and New Zealand. J. McINDOE, Dunedin, N.Z.

HOLLOS, L. 1904. Die Gasteromyceten Ungarns. Leipzig.

KAMBLY, P. E. and LEE, R. E. 1936. The Gasteromycetes of Iowa. Univ. Iowa Nat. Hist. Studies, **17**, 121–185.

LANGE, M. 1948. The Gasteromycetes of Greenland. Medd. Grønland, **147**, 1–32.

LLOYD, C. G. 1905a. The Lycoperdaceae of Australia, New Zealand and neighbouring islands, Cincinnati, Ohio.
——— 1905b. Mycological Notes 19 and 20. Lloyd Library and Museum, Cincinnati, Ohio.
——— 1906. Mycological Notes 23. Lloyd Library and Museum, Cincinnati, Ohio.
——— 1907. Mycological Notes 27. Lloyd Library and Museum, Cincinnati, Ohio.
LOHMAN, M. L. 1927. The Iowa species of *Lycoperdon*. Univ. Iowa Nat. Hist. Studies, **12**, 1–28.
MACBRIDE, T. H. and ALLIN, N. 1896. The Saprophytic fungi of Eastern Iowa. Bull. Lab. Nat. Hist. Univ. Iowa, **4**, 33–66.
MASSEE, G. 1887. A monograph of the genus *Lycoperdon* (Tournef.) Fr. J. Roy. Micr. Soc. (London), **7**, 701–727.
MORGAN, A. P. 1891. North American fungi. The Gasteromycetes. J. Cincinnati. Soc. Nat. Hist. **13**, 5–21.
PECK, C. H. 1878. N.Y. State Museum, Ann. Rept. No. 29.
——— 1879. United States species of *Lycoperdon*. Trans. Albany Inst. **9**, 285–318.
PERDECK, A. C. 1950. Revision of the Lycoperdaceae of the Netherlands. Blumea, **6**, 480–516.
PERSOON, C. H. 1801. Synopsis methodica fungorum. Gottingae.
——— 1809. Mémoire sur les Vesse-Loups ou *Lycoperdon*. J. Botan. **2**, 1–31.
SMITH, A. H. 1951. Puffballs and their allies in Michigan. University of Michigan Press, Ann Arbor, Mich.

GROWTH AND MORPHOGENESIS IN THE CANADIAN FOREST SPECIES

IV. RADIAL GROWTH IN BRANCHES AND MAIN AXIS OF PINUS RESINOSA AIT. UNDER CONDITIONS OF OPEN GROWTH, SUPPRESSION, AND RELEASE[1]

By Dorothy F. Forward[2] and Norah J. Nolan[2]

Abstract

The analysis of growth of a pine tree has been extended to include the primary branches, and this paper reports observations on radial growth, as expressed by ring width.

Trees from contrasting situations are compared, and the deliberate release of one tree from suppression permits the attribution of specific changes in growth to the change in external condition of the tree.

The primary branches provide a series of axes that automatically undergo a change in nutritional status, although the distal portions of all of them are produced simultaneously. Every primary branch is initiated at the apex of the tree and each year is overlaid by one more whorl of branches; so it advances to a relatively lower position in the tree each year, and itself adds one more internode.

The upper branches and those portions of lower ones that were formed while near the top of the tree repeat the pattern and configuration of growth in the main axis. Advance of a branch to an inferior position is associated with severe suppression and a redistribution of growth gradients.

I. Introduction

The growth of a tree is a complex phenomenon compounded of responses of its primary apical meristems and its secondary cambial meristems to both intrinsic and extrinsic forces which are not uniform either in time or in locality within the tree. In earlier publications of this series Duff and Nolan (1, 2, 3) have analyzed the components of growth of the main axis of pine trees, and have devised means of distinguishing between the influences of intrinsic and extrinsic factors. The methods of analysis proposed by Duff and Nolan have been applied by Mott *et al.* (8) to radial growth of balsam fir, tamarack, and lodgepole pine, all of which species manifest a distribution of ring width through the main trunk like that described for red pine. The observations of Onaka (9), while not complete for all internodes, are consistent with the existence of a similar pattern in *Pinus densiflora* Sieb. et Zucc. It is probable that the distribution of growth revealed by analysis of red pine is of general occurrence, at least among conifers. The principal aim of this communication and the one to follow is to describe the results of analysis of branch growth and to determine whether the same general principles evoked to explain the distri-

[1]Manuscript received September 20, 1960.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. The data reported were collected and analyzed according to a plan designed by the late Professor G. H. Duff and are an integral part of the experimental investigation of growth of the pine tree that he established. The senior author, however, assumes responsibility for the presentation of material, the conclusions reached, and the views expressed in the present paper.

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bution of growth in the main axis will also account for that in the primary branches.

During its development a branch automatically undergoes a change of status within the tree, and so affords an opportunity to trace the effect upon its growth. A pine tree produces annually one whorl of branches which subtends the currently terminal internode of the main axis, and in progression from the apex of the tree downward these whorls provide a series of branches of increasing age, and, at the same time, of changing position relative to other branches of the tree. Each branch, during its development, occupies a position in first the top and then in progressively lower whorls, and is overlaid by more and more whorls. This implies a change in nutritional status and has its association with both intrinsic and extrinsic factors. At least with respect to exposure to light, its position deteriorates, and this is accentuated in a close plantation where neighboring trees interfere with lateral illumination. Indeed the lower branches of suppressed trees lose their foliage and die, while a tree in the open remains branched and foliated almost to the ground.

The analysis that follows will show how the changing status of a branch as it develops is reflected in the growth of its successive internodes and successive rings. It will compare wood growth in trunk and branches of open-grown and plantation trees, and of one that was released from suppression when partially grown. This last tree, in particular, yields the opportunity to associate certain aspects of growth with a deliberate change in external conditions. The complete analysis of growth of both main axis and primary branches of even four trees has required such extensive treatment that it cannot all be encompassed in a single paper of manageable proportions, and the subject matter of the present paper will be restricted to a consideration of radial growth as represented by ring width. Other aspects will be dealt with in following papers.

II. Materials and Derivation of Data

1. Specimen Trees

Four trees provided the data for analysis. These were young trees, in the range of 25–30 years old, from different ecological situations and of different growth habit, as follows.

Tree K55.1 grew in the Petawawa Forest Experiment Station, Chalk River, Ontario. Although it was originally a member of a plantation, early casualties left it isolated and it grew as an individual free from interference by any neighbors. It was felled and sectioned in September, 1955.

Tree C53.5 grew near a roadside in Camp Borden, Ontario, clear of the forest by 30 ft, and 60 ft from trees in other directions. It was selected as an open-grown unsuppressed tree. When it was sectioned, however, internal evidence of a certain amount of unilateral suppression was found. This was regarded as insufficient to be of any serious consequence, but was the reason for including a second open-grown tree (K55.1) in the survey. It was cut and sectioned in September, 1953.

Tree XA57.18 was from a plantation in the North Barr Tract of the Drury forest near Craighurst, Ontario. It was situated in a plot marked out on a

southern slope where growth was maximal. The trees were planted with a 6′×6′ spacing and in 1950 serious mutual suppression was evident. The specimen tree was cut and sectioned at the beginning of October, 1957.

Tree XA58.9 was from the same plot as XA57.18 and grew under closely similar conditions until September, 1950, when that portion of the plot in which it stood was thinned to a spacing of 24′×24′ by removal of the appropriate trees. From 1950 to 1958 it grew under markedly improved conditions because of the removal of its neighbors. As the specimen tree itself and its nearest neighbors now responded by increased growth some signs of mutual interference again developed gradually, but its situation continued to be distinctly superior to that of its companion tree XA57.18, which remained in the 6′×6′ stand. Tree XA58.9 was collected in September, 1958.

II.2. Preparation of Wood Sections

When a tree was taken for analysis the general procedure was as follows. The peak of the tree with the topmost three or four whorls of branches was first removed, then individual branches, those of one whorl being kept together. Each branch was identified by a label bearing the number of the branch and the number of the whorl in which it was situated. The main axis, after being freed of branches, was taken down in portions, the internode lengths measured, and a section cut from the middle of each internode in series extending from the apex as far down the tree as internodes could be certainly identified. The severed branches were carefully denuded of their foliage, the weight of which was recorded for future use, and the branches were transported, along with the serial sections of the main axis, to the laboratory. Here the lengths of branch internodes were measured and from one to three branches from each whorl systematically sectioned for the mensuration of all their internodes. One surface of each section was polished by sanding and was ready for examination.

II.3. Measurement of Ring Width and Derivation of Data

In sections of the main axis the radius and the width of every wood ring along each of either four or eight radii were measured with a travelling microscope by direct reading from the vernier scale. "Ring width" $(r_n - r_{n-1})$ is the arithmetic mean of the four or eight individual measurements. In the sections of branch internodes the rings were too narrow for accurate measurement by this means and the ring widths were measured at higher magnification using a microscope equipped with a calibrated ocular. The radius of the pith was measured and the distance of each ring from the center of the branch section was obtained by summing the widths of all rings internal to it and adding the radius of the pith. Ring width $(r_n - r_{n-1})$ and radius (r_{n-1}) are again the means of measurements made along four or eight radii.

It would be unprofitable to repeat here any extensive explanation of the concepts employed in the analysis of tree growth based on the serial mensuration of internodes, and the reader is referred to the earlier publications in which they are described and interpreted (1, 2). However, for the sake of convenience, brief definitions of the internodal sequences to be employed are included here.

Type 1 Sequence

This is a sequence showing the values of any index of growth that are encountered in successive internodes, counting from the apex of the tree or branch, in rings laid down in any one year. Such a sequence proceeds diagonally downward and outward including for example the single ring of the first internode and the outermost ring in each succeeding internode, or in a different sequence, the innermost ring of the second internode and the second last ring formed in each lower internode. Such sequences follow the diagonal lines in a histogram of the kind shown in (1, Fig. 5). They are called oblique sequences by Mott *et al.* (8).

Type 1 sequences reveal *pattern*, which has been defined (1) as "a certain uniformity or pattern in the distribution of growth activity no matter what the circumstances of growth may be."

Type 3 Sequence

This is a sequence of values of any index of growth in successive internodes in rings laid down by internodal cambia of uniform age. Such a sequence proceeds vertically downward in a trunk or branch, for example from the single ring of the apical internode to the innermost ring of all successively lower internodes, i.e. through all internodes formed by the internodal cambium in the first year of its existence. Such sequences follow the vertical lines in the histogram of (1, Fig. 5) and consequently pass from the internodal ring laid down in the final year of the tree's growth to rings laid down in earlier and earlier years, in lower internodes. They are called vertical sequences by Mott *et al.* (8).

Type 3 sequences reveal *configuration*, which has been defined (1) as "regularity in the distribution of growth activity which, however, is not the same under all circumstances but varies with variation in such conditions as site and stand density". They reveal also *random fluctuations* or irregularities that result from climatic annual variations.

Type 4 Sequence

This is a new type of sequence which we introduce for the analysis of branch growth. It is a composite sequence involving branches in various whorls at the time of analysis, but bringing together internodal rings that existed in a particular whorl of branches at the time the ring was laid down. It is, in effect, a type 3 sequence that eliminates the effect of ultimate position of the branch on the tree by using the whorl number that is contemporary with the formation of each internodal ring instead of that which refers to the to the time of mensuration of the tree. We suggest that sequences of this type be called "contemporary whorl sequences".

III. Analysis of Ring Width

III.1. Type 1 Sequences

The ring width of trees grown under different conditions has been shown to exhibit "pattern" in the upper internodes of the main axis (1). The pattern was qualitatively of uniform character under all circumstances but subject to quantitative modification. In a suppressed tree ring width in type 1 sequences

rose to a maximum in those internodes that were in the second or third year of their existence, and declined markedly in older internodes. In an open-grown tree the maximum ring width was greater in magnitude, was achieved only in internodes in their 7th–10th year, and subsequently declined less conspicuously. In a tree whose condition gradually improved or deteriorated a corresponding change in pattern of ring widths ensued.

The first inquiry to be undertaken is whether the growth of branches reveals a similar pattern and similar quantitative modifications by a change in the conditions of growth. Pattern, since it emerged under all growth conditions observed, was believed by Duff and Nolan to be determined mainly by an intrinsic factor which was tentatively associated with a nutritional factor related to the distribution of foliage on the tree, and only secondarily by extrinsic factors, which are responsible for its modifications. A branch automatically undergoes a progressive change in its relative position in the tree as it develops and this affects both the intrinsic "nutritional" factor and certain extrinsic factors, such as light incidence. A consequence of the annual addition of whorls is that in any branch in whorl n only the ring laid down in the final year of growth grew while the branch was in the nth whorl. The ring of the previous year was laid down in whorl $n-1$, and so on, the first ring laid down in the basal internode having been produced while the branch was in the uppermost whorl of the tree. Differences in successive type 1 sequences may therefore be expected to reveal the effects of the progressive change in position of the branch. We have examined such sequences in main axis and branches of our four specimen trees and the results are set forth below.

It is manifestly impossible to show all the data accumulated in the analysis of these trees, and in all figures used in this and subsequent sections it has been our policy to select the minimum number of sequences that will illustrate a point and wherever possible to bring together data for representative branches and the main axis. Only in this way is it possible to simplify the presentation to the point where it is feasible to make convenient comparisons. Care has always been taken to select data that are representative.

Tree K55.1 (Open Grown)

Ring widths in the main axis display an obvious pattern of the conformation already described for a rapidly growing tree in the open (1, Fig. 8). Type 1 sequences of three rings of the main axis, those of 1955, 1951, and 1947 appear in Fig. 1 as solid lines. The second mode of plotting as described in (1) is employed, making the apical origins of all sequences coincide at the origin of the graph. Obvious characteristics of the pattern are broad rings, reaching a maximum width of 7–11 mm within about 7 years from the origin of the ring. The decline in older internodes is not obvious in the three sequences portrayed but in the stem as a whole it was real, though very slight.

For comparison with the main axis, the rings laid down in selected years in four branches are also shown in Fig. 1. These four branches were respectively in whorl 14 initiated in 1942, whorl 9 initiated in 1947, whorl 5 initiated in 1951, and whorl 2 initiated in 1954. Each whorl comprised 4–7 (mean 6.0) branches and only one branch was analyzed. It will appear later that, so long as obviously dwarfed branches are avoided, all branches in a whorl are similar

and any one may fairly be chosen to represent its whorl. Since the rings laid down in the branches were much smaller than those of the main axis a different ordinate scale has been used in order to reveal any pattern that exists, and the same applies to later graphs. Such a pattern is obvious in whorls close to the top of the tree. Rings laid down in branches in 1955 are marked '55 at the end of the sequence. In the upper nine whorls a pattern is obvious in the 1955 ring but in the 14th whorl from the top of the tree little or no pattern exists. This was true of the 10th to the 15th whorl, and is obviously the result of suppression due probably to shading by the upper branches. It is noteworthy that these branches without pattern arise from nodes corresponding to those internodes where the main axis shows a very slight decline in ring width.

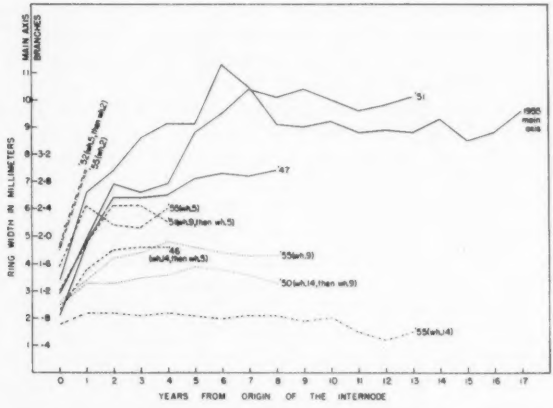

FIG. 1. Type 1 sequences of ring width for the open grown tree K55.1, plotted according to the second mode, in which the sequences are displaced on the time scale and the origins made to coincide at the origin of the abscissal scale. Solid lines represent the main axis, other lines the branches. After each sequence appears the year in which it was formed, and in parentheses, the number of the whorl in which the branch finally occurred, followed by the whorl in which it occurred when the ring was formed.

When we turn to earlier-formed rings in the same branches we find, for instance in that of whorl 14, that a definite pattern exists. But in 1950 this whorl was 9th from the top so it is comparable not with the 1955 ring of whorl 14 but with the 1955 ring of whorl 9. These two sequences are shown in dotted lines and are closely alike in pattern. Similarly the ring laid down in 1946 in what is now whorl 14 was then in the 5th whorl and is comparable with that in whorl 5 of the present tree. These are shown by broken lines along with the ring of the present whorl 9, which was whorl 5 in 1951. The final example selected is the present whorl 2 and that ring of whorl 5 that was laid down when the branch was in the 2nd whorl from the top. These show the same sharply ascending trend as the main axis in corresponding internodes.

It is clear that the branches exhibit essentially the same "pattern" of cambial activity as does the main axis provided they are near the top of the tree, but that as they are shaded by higher whorls of branches signs of suppression appear. In this open grown tree annual rings that were laid down when in the top 9 or 10 whorls exhibit "pattern"; those laid down when in lower whorls do not. In rings laid down in the 5th and 9th whorls, however, there is an indication of mild suppression in the circumstance that maximum ring width was reached in a younger internode than in the main axis.

Tree C53.5 (Open Grown)

Type 1 sequences for this tree appear in Fig. 2. The pattern of the main axis shows increasing ring width in internodes up to 6–9 years old, then a definite decline from 11 to about 15 years. Since this pattern was similar in all rings only the ring laid down in 1953 is shown in Fig. 2. For comparison five rings are chosen from a branch of whorl 18, viz. those laid down in 1953, 1947, 1944, 1940, and 1937 when this whorl was 18th, 12th, 9th, 5th, and 2nd from the top in the respective years. The 1953 ring for each corresponding whorl is also shown and again it is seen that the pattern exhibited by a branch ring is related to the position of the branch at the time it was laid down. In this tree rings formed even in the 12th whorl exhibit some pattern, but the maximum width in the branch of even the 9th node is reached earlier (second year) than in one of the 5th node (3rd–4th year), which is a sign of mild suppression. In the 18th whorl no pattern survives. Although only a few rings are shown here, the type 1 sequences for all rings of one branch from each whorl have been inspected, and these more complete records show that when about 10 (9–11) whorls exist above a branch, interference with the normal pattern becomes obvious and it is accentuated with lower position in the tree until no pattern exists.

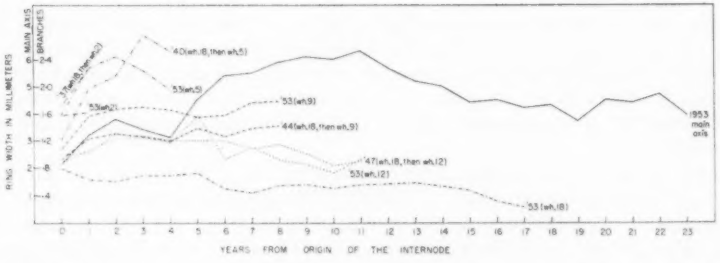

FIG. 2. Type 1 sequences of ring width for the open-grown tree C53.5. The solid line represents the main axis, other lines the branches. For further explanation see Fig. 1 and text.

Tree XA57.18

This was a typically suppressed tree with only nine foliage-bearing whorls of branches. The pattern of ring width in the main axis is that of a suppressed tree as described earlier (1, Fig. 4) with maximum width of 5–7 mm reached at 2–4 years from the origin of the internode, and a long decline to a level

phase at 7–12 years. The two sequences plotted in Fig. 3 fall within these limits. Amongst the branches it is clear that those rings that were laid down when the whorl was 3rd or 4th from the top show a definite pattern; but in lower whorls, of which the 6th and the 9th are represented here, the pattern does not develop. The branches of these lower whorls would suffer more severe shading than the corresponding branches of an open grown tree because of interference from surrounding trees, so it is not surprising to find that only the upper 4 or 5 whorls rather than 9 or 10 as in open-grown trees show no interference with the pattern of cambial activity.

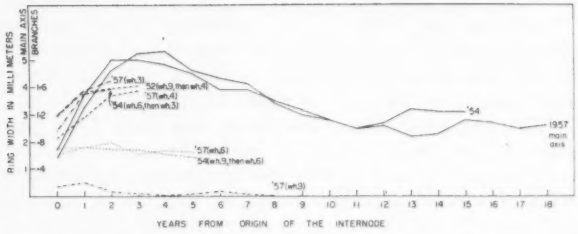

FIG. 3. Type 1 sequences of ring width for the suppressed tree XA57.18. Solid lines represent the main axis, other lines the branches. For further explanation see Fig. 1 and text.

The suppressed tree therefore differs from the open-grown trees not only in reaching maximum ring width in the main axis within 3 years from the origin of the internode, but also in showing a definite pattern only in the rings laid down by branches when they are in the top four whorls of the tree.

Tree XA58.9

This was a companion tree to XA57.18 in a close plantation until it was released at the end of the 1950 growing season. It was analyzed 8 years after its release. Ring width was measured in the main axis and in several branches from each whorl. Here, in order to show adequately the effects of release, it is necessary to plot separately (Figs. 4, 5, and 6) the sequences of trunk and branches.

Figure 4 shows type 1 sequences in the main axis for the rings of alternate years in the period up to 1950, and from 1951 onward. Broken lines represent sequences in rings laid down before release of the tree, solid lines those laid down after its release. It is clear that from 1946 to 1950 the effects of suppression were increasing and in the latter year a maximum ring width of 5.9 mm was reached in the 3-year-old internode. The next year after release, ring width increased for at least 10 internodes downward from the apex and, in the 11th, reached a value of 11.3 mm, nearly double the maximum of the preceding year. The 1950 sequence is typical of a suppressed tree while the 1951 sequence is typical of a tree in the open. The sequences of later years suggest that as the tree, now centered 24 ft from its nearest neighbors, responded by increased growth while its neighbors presumably did the same, mutual interference gradually came to be felt again and a regression in pattern

developed, continuing until the tree was collected in 1958. The ring sequence of 1958, while still not that of a suppressed tree, had a lower maximum ring width than that of 1951, and this was reached in a younger internode.

Many branches of this tree were analyzed. The whorls were composed of four to eight branches (average number 6.4), and except in the very lowest whorls where some branches were dead, at least three from each whorl were sectioned and examined. This permits us to judge whether one branch fairly represents a whorl. Comparison of the type 1 sequences of three branches

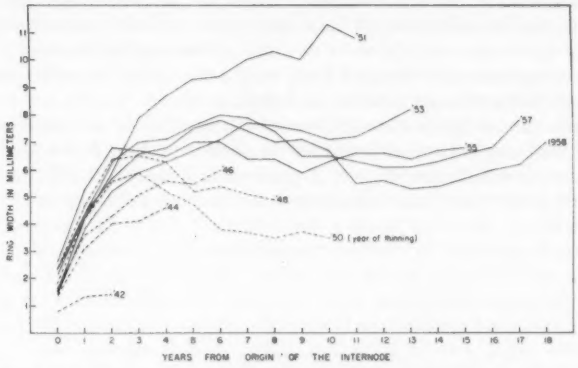

FIG. 4. Type 1 sequences of ring width in the main axis of the released tree XA58.9. Broken lines represent rings laid down before release of the tree, solid lines rings laid down after release. For further explanation see Fig. 1 and text.

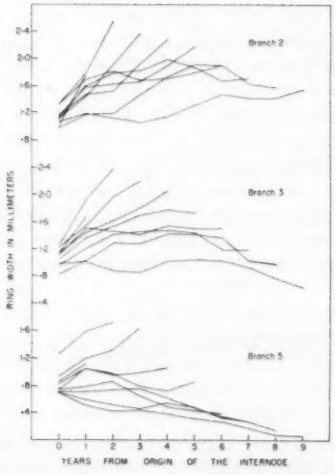

FIG. 5. Type 1 sequences of ring width from three branches of whorl 10, from tree XA58.9. The upper two represent typical branches and the lower one a dwarfed branch.

from many whorls makes it quite plain that in all branches of a whorl they agree remarkably well in form and magnitude, with very few exceptions. All the exceptions were of the same kind, and referred to singly occurring dwarfed branches with very little foliage which were deliberately chosen for analysis in order that they might be compared with typical branches. Figure 5 illustrates the ring width pattern in three branches of whorl 10, of which branches 2 and 3 were typical and branch 5 was dwarfed. The families of sequences belonging to branches 2 and 3 are closely similar in almost every detail, while that of branch 5 shows abnormally narrow rings and abnormal pattern. It was equally true of this and other whorls that, apart from the dwarfs, any one branch would serve as a fair representative and in the analyses that follow sequences are selected from only one typical branch in any whorl, and dwarfed branches are always excluded.

Even with this simplification it would be impossible to reproduce all the data here, but a selection of branches is shown in Fig. 6. In the top seven whorls, three of which are shown, a pattern is evident in all the type 1 sequences and it is of the kind characteristic of trees growing in the open, i.e.

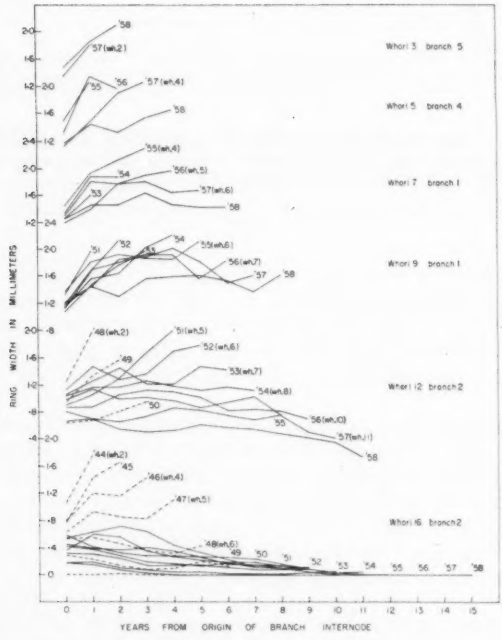
RING WIDTH IN MILLIMETERS
YEARS FROM ORIGIN OF BRANCH INTERNODE
Whorl 3 branch 5
Whorl 5 branch 4
Whorl 7 branch 1
Whorl 9 branch 1
Whorl 12 branch 2
Whorl 16 branch 2

FIG. 6. Type 1 sequences of ring width in the branches of the released tree XA58.9. Broken lines represent rings laid down before release of the tree, solid lines rings laid down after release. In a column at the right hand side of the figure are the numbers of the whorls when the tree was cut. In parentheses, following the year of formation of each ring is the number of the whorl in which the ring was laid down.

the ring width increases for several internodes down the branch from its tip. In whorl 9 the ring laid down in 1958 shows some modification of pattern, and it is characteristic of open grown trees that the pattern is partially suppressed in whorls that are overtopped by as many as eight whorls when the ring is formed. But this is true only of the ring laid down in 1958 in this whorl. The ring laid down in 1957 was then in the eighth whorl, and has a typical pattern. Similarly the rings laid down in earlier years were nearer to the top of the tree when they were formed, and all are patterned. In this figure the year of production of the ring appears at the end of each sequence and in parentheses after it is the number of the whorl in which it was situated at the time of its formation.

When we come to whorl 12 a new factor appears, because here we have rings laid down before release of the tree as well as after this change in its condition of growth. Here we find decreasing ring width in the rings formed in 1948–50 (shown as broken lines) with little evidence of pattern in the ring produced in 1950 although it was then in the 4th whorl from the top of the tree. In 1951, however, when the tree showed the maximum effect of liberation, the ring laid down in this branch showed a large increase in width from the first to the 5th internode i.e. it exhibited a pattern of the type found in open-grown trees. This branch was then in the 5th whorl from the top of the tree. As we progress to the rings laid down in later years and in lower whorls, we find that in 1955 when it was in the 9th whorl the pattern had been completely suppressed. This corresponds to what occurred in the 10th whorl of tree K55.1 grown in the open. It is clear therefore that this branch, like the main axis, changed the character of its cambial growth from that of a suppressed tree to that of an open-grown tree after the plantation was opened up from a 6′×6′ spacing to one of 24′×24′ after the 1950 growing season.

In whorl 16 the branches formed when they were within eight whorls of the top of the tree were all laid down before liberation of the tree and here only those in the top five whorls at the time the ring was formed exhibit pattern. In the 1948 ring (then in whorl 6) the pattern is completely suppressed, as one would expect of a tree in a 6′×6′ plantation. By 1951 after release of the tree, the branch represented by this series of graphs was already in the 9th whorl where pattern is beginning to be suppressed even in an open-grown tree, and the rings laid down in this and subsequent years show little or no pattern. These were all low on the tree and increasingly shaded by surrounding trees, and although the tree as a whole was now in a more open situation they were unable to respond because of intratree suppression by the overlying whorls of branches.

The conclusions derived from a detailed analysis of these few branches were borne out by the data from other branches and may be stated in a more general form that applies to the tree as a whole. In rings that were laid down before release of the tree, pattern disappeared in a branch when it was overtopped by more than four or five whorls. In rings laid down after release of the tree, pattern persisted in branches that were not displaced from the apex of the tree by more than seven to nine whorls. This tree therefore demonstrates that liberation from suppression not only causes the inherent pattern of growth in the main axis to change from the kind characteristic of a sup-

pressed to that of an open-grown tree, but also it brings about corresponding changes in those branches that benefit from the release.

III.2. Discussion of Type 1 Sequences

The four trees from contrasting situations reveal plainly that branches show a pattern of cambial activity just as does the main axis, but they also reveal that an important factor affecting pattern is the serial position of a branch on the tree. Only in the upper whorls, more or fewer according to the degree of suppression of the tree as a whole, is pattern exhibited, and in branches of lower whorls cambial activity is restricted and ring width is consistently small in all internodes. In effect this means that even in an open-grown tree the lower branches are suppressed and pattern disappears.

In the main axis of trees growing under a wide range of external conditions the essential form of the pattern of ring width is manifested in a rising and then falling magnitude as one proceeds downward from the apex through the upper internodes of the trunk. It may be quantitatively modified but it is always present. In the lower branches of both suppressed and open-grown trees, however, it is non-existent, and this is the only situation met with in our investigation where pattern is obliterated. It is therefore of considerable interest to attempt to characterize the differences between the distal portion of a lower branch and that of the main axis in the hope of gaining further insight into the biological determination of patterned growth.

Duff and Nolan (1) tentatively ascribed the source of the pattern of cambial activity to a gradient in its nutritional status which, in turn, depends on a gradient in the amount of effectively lighted foliage that is supplying organic nutriment to the distal internodes of the tree. They point out that within a type 1 sequence, progressing from its origin at the apex, the successive rings that make up the sequence lie farther and farther from the apex of the tree and that increase in cambial activity as expressed in ring width occurs in the conical portion of the tree where the total amount of foliage on branches subtending the successive internodes is also increasing basipetally. Since, as Duff and Nolan recognized, there is also a gradient in the age of the cambia producing successive rings in the sequence, this presents itself as an alternative or participating determinant of patterned growth. The data for main axes afford no means of distinguishing between the effects of age and nutrition, but those for the branches do afford such means.

The chronological age of the terminal internodes of all branches is the same as that of the terminal internode of the main axis,—all were produced in the final year of the tree's growth at a time equidistant from the origin of the active shoot during germination of the seed. And every branch presents a similar age gradient as one progresses downward from the apex in a type 1 sequence. Yet in the upper branches and the main stem the cambial growth is patterned but in the lower branches it is not. It therefore becomes apparent that the age of the cambium per se is not the determining factor in the causation of pattern. On the other hand the distal portions of lower branches differ markedly in nutritional status from the distal portion of upper branches and the main stem. The most obvious difference in the external situation is in exposure to light. Even in the closest stand the upper few internodes of the

main trunk are subtended by branches whose foliage is exposed to light from above, and the same is true of the uppermost branches. But lower branches even in a tree growing in the open are subjected to shading by the upper whorls. The distal portion of a lower branch does not with its subordinates achieve the same conical form that is characteristic of the distal portion of the tree, and this is related to the circumstance that the branch suffers from some degree of shading throughout its length. The very strong probability is that no gradient in nutritional status of the cambium similar in steepness to that of the trunk and upper branches exists in the lower branches. Data are at hand for an enquiry into the distribution of foliage in individual branches and in the tree as a whole, so that some experimental evidence can be brought to bear on this problem in the future. This will show that so far as amount of foliage is concerned gradients are strongly modified by position on the tree, but the examination of possible correlation with patterned growth must be postponed until a later date. For the time being it may only be said that while the data under present consideration appear to rule out age of the cambium as a controlling factor, nutritional status of the cambium remains a probable determinant of the pattern of cambial growth.

Duff and Nolan (2) do not exclude from their "nutritional" factor the supply of minor essential organic substances, but it is usual to distinguish between "nutritional" and "hormonal" control of cambial growth. It does not seem profitable to set these up as opposing theories since both nutrition and hormone supply must play their roles in growth, but one would hope eventually to be able to define these roles more clearly than is now possible. So far as the present evidence from branch analysis is concerned, it demonstrates that displacement from the apical meristem of a branch is not the dominant factor in determining cambial growth, but that position of the branch on the tree is an overriding factor. Distinction between a "hormonal" and a "nutritional" factor would require a knowledge of the hormonal status of the apices of individual branches that is not available. Data on the relationship of buds and foliage to cambial growth are, however, on hand and will be discussed in a later publication.

III.3. Type 3 Sequences

The third type of ring sequence described by Duff and Nolan (1) consists of successive internodal rings laid down by cambia of uniform age, and consequently laid down when in a constant position relative to the apex of the tree or of a branch. Such sequences afford an opportunity to observe the configuration impressed upon a tree by the stable characteristics of its site, as well as the fluctuations superimposed by random annual variations. This type of sequence serves also to reveal the gradually changing condition of an individual branch as its position on the tree is affected by the development of successive branches above it, and it is relegated to a lower and lower relative position on the tree.

In the present section are presented type 3 sequences for branches from representative whorls, for comparison with those of the main axis, in the same four trees of different condition whose *pattern* we have examined in the preceding section.

Tree K55.1—Open Grown

In the main axis of this tree the configuration is expressed as a general upward trend in ring width during the first 8 or 10 years of the time range and little or no further change until 1955. Random variations are superimposed and these are most pronounced in the rings that were initiated in the earlier years. Figure 7 includes four type 3 sequences from the main axis, two initiated in the early years in the second and fourth position from the pith, together with two later formed sequences. Figure 7 also shows a selection of type 3 sequences from branches in different positions on the tree. All are plotted according to the second mode, which in this type of sequence, places

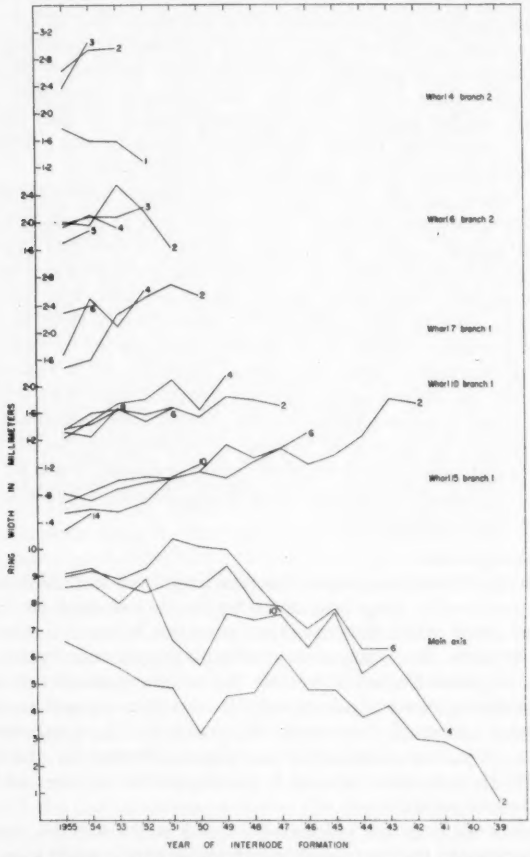
RING WIDTH IN MILLIMETERS
YEAR OF INTERNODE FORMATION
1955 54 53 52 51 50 49 48 47 46 45 44 43 42 41 40 39
Whorl 4 branch 2
Whorl 6 branch 2
Whorl 7 branch 1
Whorl 10 branch 1
Whorl 5 branch 1
Main axis

FIG. 7. Type 3 sequences of ring width for the open-grown tree K55.1, plotted according to the second mode. The number at the end of each sequence represents the position of the sequence as measured from the pith, and at the same time the number of the internode in which it originated and the age of the cambium (in years) when it was formed.

vertically above one another all internodal rings formed in 1 year. Each sequence is numbered to show its position as counted from the pith, and therefore also the number of the internode in which it originated. The longest sequence of a group is in most instances the second from the pith, which means that it was initiated in the second internode from the apex of the branch. Shorter sequences are for rings which lie further from the pith and which first made their appearance in lower internodes. These are the vertical sequences in a histogram such as that illustrated in (1, Fig. 5). The sequences are shorter in the higher whorls of branches because these were initiated later in the growth of the tree and have fewer internodes than lower branches.

It is at once apparent that the position of the branch on the tree affected the configuration. In the 10th and lower whorls, all cambia, of whatever age, produced rings that show a similar downward trend in width from the time of origin of the sequence to 1955. In whorls 7–9 this is less uniform but the downward trend is unmistakable, while in whorl 6 it is non-existent, and in higher whorls it does not appear in rings formed by 1-year-old cambium but does in those formed by older cambia.

This downward trend, where it appears, introduces a difference between the branches and the main axis. The explanation lies in the circumstance that rings in the successive internodes of a type 3 sequence are produced in different years and at times when the particular branch in which they are formed is in a different position relative to the apex of the tree. Branch 1 of whorl 15 may be used to illustrate this. In sequence 2 each internodal ring was laid down when the branch internode was the second one from the apex *of the branch*, but in 1942 this branch was in the second whorl and was overtopped only by the young whorl developing in the current growing season, while in 1948 the same branch was in the 8th whorl from the top of the tree and in 1955 it was in the 15th whorl. Therefore, although the successive rings of sequence 2 were always laid down in the second internode of the branch, they occupied a lower and lower relative position on the tree. This necessarily introduces a new factor in the environment of the individual branch which, in spite of maintenance of favorable conditions or even improving conditions for the tree as a whole, finds itself in progressively deteriorating conditions because of shading by more and more overlying whorls of branches. It is only in the topmost branches of the tree that this factor does not come into play. The main axis differs in that its second internode is always at the top of the tree and consequently in the same nutritional state in succeeding years provided that no major change in site conditions intervenes. Therefore although the branches of this tree differ from the main axis in configuration they do so in such a way as to indicate conformity to the general principles formulated for the main axis (1). Configuration is expected to reveal the influence of external factors, and the downward trend in the type 3 sequences of branches which are individually subjected to increasing suppression as the tree grows, is just what one would expect to find in the main axis of a tree undergoing gradual suppression. It was shown in section III.1 that in this same tree, "pattern" as revealed in type 1 sequences survived in the upper 9 or 10 whorls of branches. But pattern is less sensitive to environment and is probably dependent, rather, on an intrinsic factor.

Tree C53.5—Open Grown

The configuration of the main axis of this tree is represented in Fig. 8 by three type 3 sequences only, while the description that follows is based on examination of the whole series of sequences. An upward trend in width of rings in those internodes formed between 1930 and 1942 is then replaced by a decline to 1949 with no considerable change thereafter until the tree was felled in 1953. In the branches the trends are essentially the same with the exception that in the older branches, represented by whorl 22 branch 2, ring width was declining during a period of years (1936–42) when it was increasing in the main axis. The apparent conformity in later years may therefore be fortuitous and evident only because the configuration in the main axis was a declining one when most of the branches were growing. In this tree it is consequently impossible to segregate the effect upon the branches of localized suppression within the tree from that occasioned by the condition of the tree as a whole, except in whorl 22 where sequences 2 and 4 declined from the time of their origin until 1942, while ring width was increasing in the main axis.

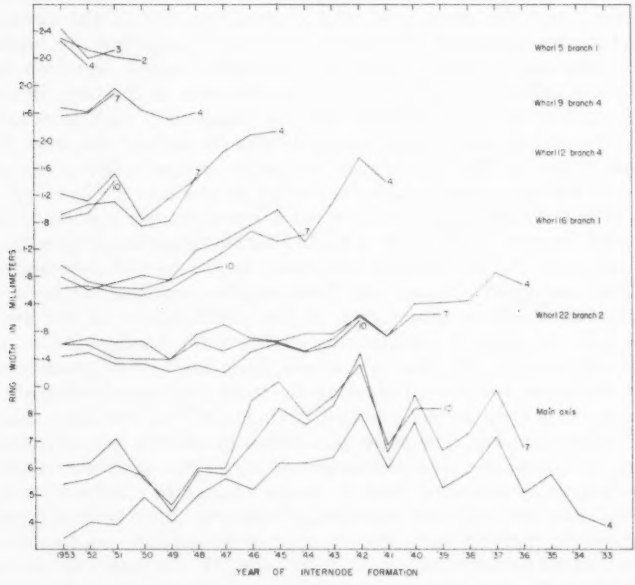
Whorl 5 branch 1
Whorl 9 branch 4
Whorl 12 branch 4
Whorl 16 branch 1
Whorl 22 branch 2
Main axis
RING WIDTH IN MILLIMETERS
YEAR OF INTERNODE FORMATION
1953 52 51 50 49 48 47 46 45 44 43 42 41 40 39 38 37 36 35 34 33

FIG. 8. Type 3 sequences of ring width for the open-grown tree C53.5. For explanation see Fig. 7 and text.

Tree XA57.18—Suppressed in a 6′×6′ Plantation

In this plantation tree the configuration of type 3 sequences in the main axis indicates improving growth in the earliest years when the tree was small and presumably relatively free of interference from its neighbors. This was

followed by an over-all decline in cambial activity after interference developed. This configuration appears in Fig. 9 as a steep rise from ring width of 1 mm or less in 1940–41 to 4–5 mm in 1944, followed by a long period of little change or a slight decline to 1954–57. A conspicuous depression in ring width in 1949 with recovery in 1950–51 was recorded also in other trees of the Craighurst plantation (cf. Fig. 10 and (1), Fig. 21), and it reflects climatic conditions at the site in those years.

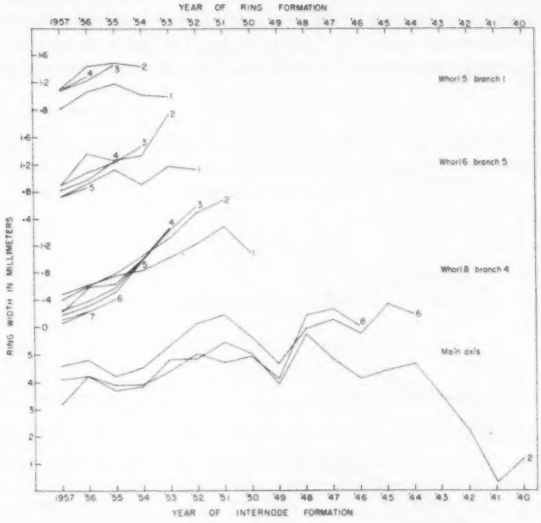
YEAR OF RING FORMATION
1957 '56 '55 '54 '53 '52 '51 '50 '49 '48 '47 '46 '45 '44 '43 '42 '41 '40
RING WIDTH IN MILLIMETERS
Whorl 5 branch 1
Whorl 6 branch 5
Whorl 8 branch 4
Main axis
YEAR OF INTERNODE FORMATION

FIG. 9. Type 3 sequences of ring width for the suppressed tree XA57.18. For explanation see Fig. 7 and text.

Branches from the nine foliage-bearing whorls were examined and those of the top five, initiated in 1953 or later, showed no consistent drift in ring widths in the type 3 sequences. This corresponds to the contemporary rings of the main axis and indicates no intratree suppression in these five whorls. In whorl 6 a definite decline was evident in all sequences except the peripheral one, i.e. sequence 1, initiated in 1952. Since all rings belonging to sequence 1 were laid down while in the terminal internode of the branch as it advanced from the first to the sixth whorl, and since this branch was the first to show any indication of being overshadowed, it is perhaps not surprising that this sequence is free from signs of intratree suppression. Branches of the lower whorls, 7–9, however, showed a consistent and steeper decline in ring width extending through all their internodes from the time of their inception until 1957. It is reasonable to suppose that this modification is of the same origin as that proposed for the open-grown tree, K55.1, viz. suppression occasioned by the annual addition of a whorl of branches above each individual branch that was analyzed.

Tree XA58.9—Released from Suppression

The release of this tree at the end of the 1950 growing season serves to demonstrate that the configuration of cambial activity in the branches as well as in the main axis is under the influence of extrinsic factors. Figure 10 shows type 3 sequences of the main axis and of representative branches. One obvious difference from the comparison tree that remained in the close plantation is the survival of 18 whorls of foliage-bearing branches in contrast to only nine such whorls in tree XA57.18. Another is the marked increase in ring width of both main axis and branches after the release of the tree.

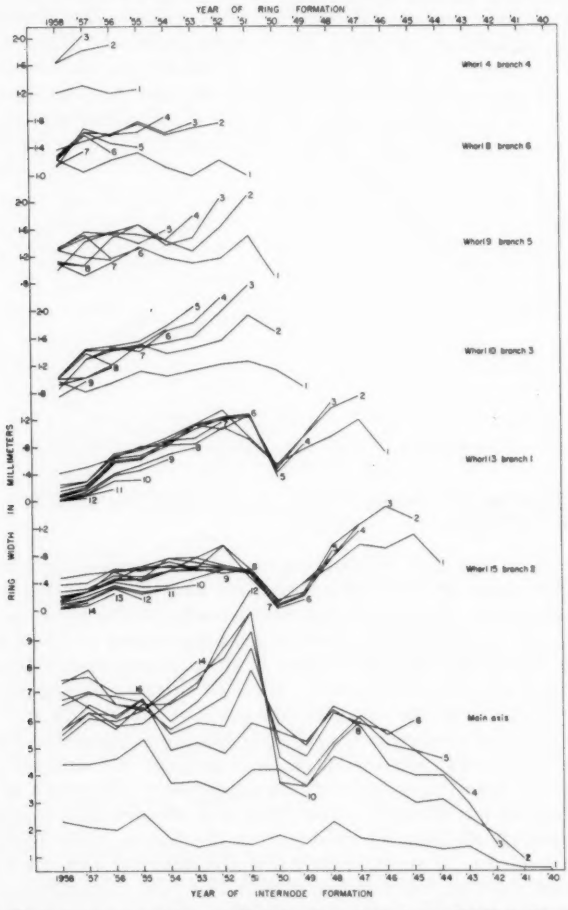
YEAR OF RING FORMATION
1958 '57 '56 '55 '54 '53 '52 '51 '50 '49 '48 '47 '46 '45 '44 '43 '42 '41 '40
Whorl 4 branch 4
Whorl 8 branch 6
Whorl 9 branch 5
Whorl 10 branch 3
Whorl 13 branch 1
Whorl 15 branch 2
Main axis
RING WIDTH IN MILLIMETERS
YEAR OF INTERNODE FORMATION

FIG. 10. Type 3 sequences of ring width for the released tree XA58.9. For explanation see Fig. 7 and text.

The configuration of type 3 sequences in the main axis conforms very closely to that of its companion tree XA57.18 up to 1950 while both were in the same plantation and had the same spacing. In 1951, the first year of growth following the operation of felling trees to make a 24′×24′ spacing around tree XA58.9, all but the three innermost sequences show a large increase in ring width which is now of the order of magnitude (8–10 mm) found in the open-grown trees already described (Figs. 7, 8) and much greater than that in XA57.18 (Fig. 9). Within the series of sequences from the 4th to the 11th, response was not uniform but increased toward the outer sequences which were formed by older cambia and initiated in lower internodes of the main axis. Table I shows, for several sequences, the percentage increase in ring

TABLE I

Ring widths, in mm, in the main axis of released and suppressed trees

	Released tree XA58.9					
Sequence No.	4	5	6	7	10	11
Ring width 1950	5.9	5.2	4.7	3.8	3.7	3.5
Ring width 1951	7.9	8.7	9.3	9.4	10.0	11.3
% increase in 1951	34	67	98	147	170	223
	Suppressed tree XA57.18					
Sequence No.	4	5	6	8	10	11
Ring width 1950	6.8	6.4	5.6	4.9	4.9	5.0
Ring width 1951	7.3	6.8	6.4	4.7	4.7	4.8
% increase in 1951	7.4	6.3	14	−4.1	−4.1	−4.0

width in 1951 over that of 1950. This rises conspicuously as one proceeds to outer sequences, and it is clear that the rise depends on a reversal in order of ring widths which in 1950 were progressively lower in outer sequences, in 1951 progressively higher. From Fig. 10 it may be seen that the differential response persisted until, in 1955, ring widths of all sequences from the 3rd to the 16th fell within a narrow range. This reversal of gradient in the width of rings laid down by cambia of increasing age resulted from improving the exposure of the tree, and is equivalent to the modification of pattern already seen in type 1 sequences. It provides additional evidence that the age of the cambium is not itself the main determinant of cambial activity. On the other hand it is undoubtedly related to the fact that the internodal rings laid down in 1951 in outer sequences were lower in the trunk and were nourished by those branches that benefited from release of the tree.

The lower whorls of branches that were initiated before 1950 reveal a marked increase in ring width in their 1951 internodes over that of 1950. In the preceding years (1945–50) the branches of whorls 13 and 15 had already begun to show the internal suppression resulting from the deterioration of their position in the tree and show a downward trend throughout this period while the main axis essentially maintained its growth, though exhibiting annual fluctuations. In 1951 whorls 15 and 13 were the 8th and 6th whorls from the top of the tree. Release of the tree improved the exposure of branches to lateral light as well as removing other kinds of competition, and the branches responded by a large increase in ring width which brought them, like the

rings of the main axis, to the order of magnitude characteristic of corresponding branches of open-grown trees, e.g. they increased in whorl 13 from less than 0.5 mm in 1950 to the order of 1–1.2 mm in 1951.

After this sudden increase the characteristic downward drift was resumed in branches of the lower whorls and continued until 1958 but less steeply than during the years of suppression before 1950. This was evident in all whorls from 10 downward, although in whorl 10 the ring formed by 1-year cambium, i.e. in the terminal internode of the branch, failed to show the drift. Whorl 9 was transitional, showing some indication of decline, but in higher whorls no consistent decline was evident. The effect of release of the tree on the suppression of lower branches may be seen by comparing whorl 8, branch 6, in tree XA58.9 (Fig. 10) with whorl 8, branch 4, of tree XA57.18 (Fig. 9). Both trees in 1951 laid down in the first branch internode (sequence 1) rings of 1.0–1.3 mm in width. In 1957 the released tree again laid down in its 1st internode (sequence 1) a ring of more than 1.2 mm in width, while the suppressed tree formed a ring only 0.5 mm wide. In 1951–53 both trees laid down rings about 1.8 mm wide in the second and third internodes of the branch (sequences 2 and 3) when they were initiated. In 1957 the released tree produced rings in those internodes of 1.1–1.4 mm width, while in the suppressed tree they were 0.2–0.4 mm wide. This means that at the branch tips near the periphery of the tree, branches of the 8th whorl in the released tree showed little effect of shading by higher whorls, whereas, in the tree closely encompassed by neighbors, even the tips of branches in the 7th–9th whorls showed marked signs of suppression. The range of ring widths laid down in a branch of the 8th whorl in 1957 was 1.1–1.4 mm in the released tree, 0–0.5 mm in the suppressed tree.

III.4. Discussion of Type 3 Sequences

In summary, a comparison of configuration in the branches with that in the main axes of the four trees shows that only in the upper whorls do the two conform, while in lower branches the configuration is expressed as a consistent increase backward from the branch apex. This results from the circumstance that cambia of uniform age in more basal internodes of a branch produced their internodal rings when the branch was nearer to the apex of the tree, and reflects the influence of position on the tree upon configuration in a branch. This means that an intrinsic factor affects configuration in the branches. That it is also subject to external influences is manifested by the annual fluctuations that correspond in general to those in the sequences for the main axis, and by the response of both main axis and branches to a deliberate change in the external conditions of one tree (XA58.9).

There have been frequent observations of increased trunk diameter of trees in response to thinning of stands. Those of Engle and Smith (4) refer to red pine. In a 10-year period after thinning, diameter growth greatly exceeded that in unthinned controls. Holmsgaard (7) recorded increased ring width following light thinning of stands of Norway spruce, oak, ash, beech, and alder. The response was of small magnitude near the base of old trees under his conditions, but it was general for widely different genera of both coniferous and deciduous trees. It has also been observed that branches

share in the improved radial growth of trees with wide spacing as compared with those in closer stands. For instance, Eversole (5) found basal diameter in lower branches of Douglas fir, as did Guillebaud (6) with Norway spruce, to be greater in trees with wider spacing.

In our analysis of tree XA58.9 we present a more comprehensive account of the effects of release on the growth of a tree than has hitherto been attempted. It reveals that the response of cambial activity is immediate and that it is not uniform throughout the tree. Increased diameter after a period of years would be the accumulation of improved growth each year after release. In tree XA58.9 we see that ring width was actually maximum in certain sequences in the first year after release. Those branches in the top whorls that had originally been well exposed showed no change in cambial growth after the removal of neighboring trees, and the same was true of the uppermost internodes of the trunk. Branches that had been suppressed but were in a sufficiently favorable position to grow well in an open-grown tree, responded by a marked increase in their cambial activity, and the subtending internodes of the main axis showed a progressive increase in cambial activity as the number of well-grown branches above them increased.

In an earlier section attention was called to the obliteration of pattern in type 1 sequences of lower branches. The corresponding evidence of suppression as revealed by type 3 sequences is the consistent decline in width of internodal rings formed by cambia of uniform age as they find themselves in lower and lower whorls of branches.

III.5. Type 4, or "Contemporary Whorl", Sequences

We have seen that type 3 configuration in the branches has not the same biological meaning as in the main axis, but is influenced by a progressive change in relative position of the branch on the tree as consecutive internodes develop. For the growth of branches, therefore, a new type of sequence is introduced. This is a collective sequence for existing branches. It is a modification of type 3, but instead of including the series of internodes of one existing branch at the time of analysis it brings together those internodal rings that were laid down when the branch was in a particular whorl as counted from the apex of the tree, although these internodes later came to be in different whorls.

Such sequences for tree XA57.18 are shown in Fig. 11A. The sequence of whorl 1 includes widths of all those rings that were laid down when the branch in which they occurred was in the topmost whorl of the tree. The first point in the sequence represents the ring laid down in 1957 in what was finally the first whorl; the second point is for a ring in the whorl that was topmost in 1956, although when the tree was cut in 1957 it was in the second whorl. The sequence proceeds back to 1949 including only rings laid down when in the first whorl of branches. These were, of course, the innermost rings in the basal internodes of existing branches in 1957. Each point in this and all other sequences of Fig. 11 is actually an average of widths of corresponding rings in two or three branches from one whorl.

The next pair of sequences (whorl 2) were laid down when in the second whorl. Two sequences are involved here. That labelled 1 was formed in the

contemporary first internode of its branch by cambium in its first year of existence; that labelled 2 was formed in the second internode of its branch by cambium in its second year. The groups of sequences exhibit configuration in internodes formed when in branches of particular whorls whose serial numbers appear in Fig. 11. An individual sequence in a group, numbered from the pith of the branch internode, shows also the serial number of the branch internode when the internodal ring was laid down.

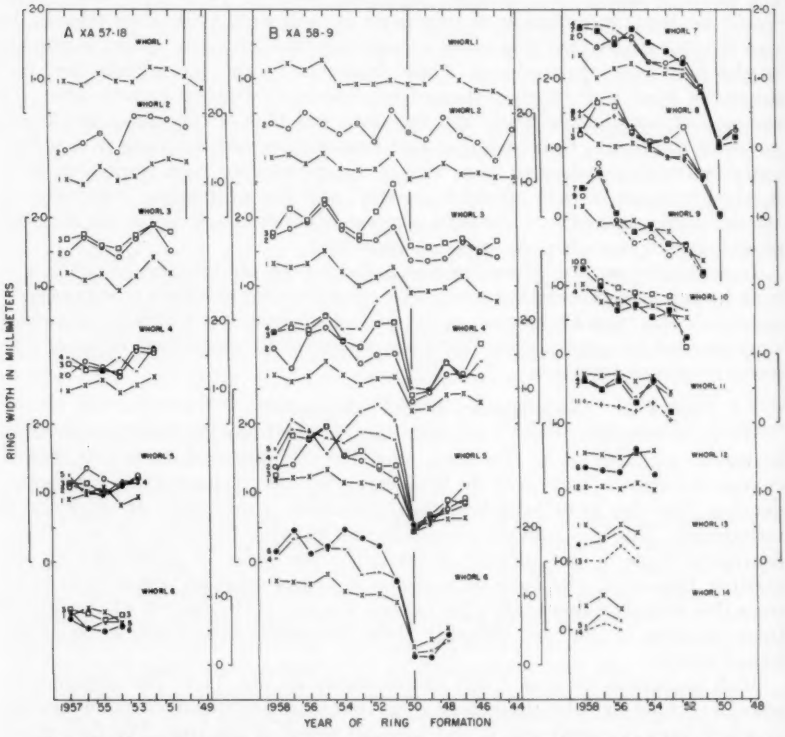

FIG. 11. Type 4 sequences of ring width for the suppressed tree XA57.18 and the released tree XA58.9, plotted according to the second mode. The number at the end of each sequence represents the position of the sequence as measured from the pith of a branch internode and at the same time the serial number of the branch internode in which it originated and the age (in years) of the cambium that produced it. The whorl number shows the position of the whorl, counted from the apex of the tree, in which any internode occurred at the time when an internodal ring in any sequence was laid down.

It now becomes clear that the downward slope of type 3 branch sequences with time (cf. Fig. 9) is largely the result of the fact that in succeeding years consecutive internodal rings in a sequence were produced in a branch overtopped by more and more whorls. The very gentle downward slope from 1951

to 1957 of type 4 sequences in Fig. 11A corresponds more closely to the configuration shown by the main axis in the type 3 sequences of Fig. 9 than to the steeply sloping type 3 sequences of the branches.

Figure 11B sets out type 4 sequences for tree XA58.9, the companion tree released in 1950. For the 6th and lower whorls some of the sequences are omitted, and only enough of them have been included to show the effect of internode position within the branch. In this tree, also, the configuration of type 4 branch sequences corresponds more closely to that of type 3 sequences of the main axis, as shown in Fig. 10, than to the sloping configuration of type 3 branch sequences. This is the effect of eliminating from individual sequences the changing relative position of the branch on the tree.

In order to draw attention to the effects of release, vertical lines have been drawn in Fig. 11 to mark the year 1950. Liberation of the tree had no major effect on the width of rings laid down in branches when in the first two whorls, and, in the third whorl, only in sequence 3 is the increase from 1950 to 1951 greater than random fluctuations in other parts of the sequence. Internodal rings laid down in branches when they were in the 4th to 8th whorls showed the greatest effect of release. Rings laid down in the 9th and 10th whorls in 1951 were narrow, but in later years rings that were laid down in this position were distinctly wider. Those laid down when in the 11th and lower whorls were always narrow and never achieved a width of much more than 0.5 mm.

In the 3rd–5th whorls a differential response in internodal sequences is conspicuous and the array of type 4 sequences in these positions is closely similar in configuration to that in the main axis (cf. Fig. 10), rings laid down in the more basal internodes in a branch showing the greater increase in width. In the lower whorls whose cambial growth remains suppressed the order of the sequences is reversed and rings initiated in the basal internodes of basal branches make scarcely measurable growth.

The true effect of intratree suppression on ring width can be seen by comparing the groups of sequences in different whorls in Fig. 11, and the effect of a change in external conditions can be seen in the sequences for individual whorls without the complicating factor of changing relative position of a branch on the tree as its successive internodes are formed. We now see clearly that the marked rise in type 3 sequences of branches from the lower whorls (e.g. whorls 13 and 15, Fig. 10) occurred after release of the tree because the internodal rings laid down in 1951 in these branches were then in a favorable position on the tree after its release. They were laid down when in the 6th and 8th whorls.

In summary, the uppermost whorls were but little affected at any time by closely surrounding trees and never suffered from suppression; the internodes that were already in branches of the 10th or lower whorl when the tree was released were then suffering from intratree suppression and continued to be suppressed by an increasing number of overlying whorls after 1951. It was only those internodes that in 1951 occurred in branches of the 4th to 8th whorls, initially suppressed, that were in a position to make a maximal response to release, for in a close plantation the 4th to 8th whorls may suffer suppression but in the open they do not.

IV. General Discussion

Analysis of ring width in trunk and primary branches constitutes one step toward analysis of the integrated growth of the tree. Increase in length, circumference, wood volume, and cambial area will be considered in the following paper, and the main discussion of biological controls will be postponed until the more complete data have been presented.

Duff and Nolan (1) introduced two types of longitudinal sequence in addition to the radial sequence of annual rings for analyzing the growth of a tree trunk. The first of these reveals the *pattern* of cambial growth which is primarily under intrinsic control, and their type 3 sequence reveals *configuration* which is subject to extrinsic control.

The first type of sequence applies equally well to branch growth and shows a patterned growth in the upper branches of a tree that conforms to that of the main axis. But lower branches that are heavily shaded by upper branches or by neighboring trees, or both, fail to develop a pattern of cambial growth. This is the only situation in which pattern has not been observed in a completely analyzed trunk or branch. The distal internodes of these lower branches are formed simultaneously with those of upper branches and the main axis, and the absence of pattern serves to show that it is not the age of the cambium nor distance from an apical meristem that is the primary determinant of cambial activity. The absence of pattern, is, however, undoubtedly related to the heavy shading to which the lower branches are subjected even in an open-grown tree, and this leaves open the probability that nutritional status is an important factor in producing patterned growth.

The configuration of ring width in type 3 sequences in the branches except in well-exposed branches near the top of the tree does not conform to that in the main axis. This is because an intrinsic factor here enters into the determination of configuration as defined by Duff and Nolan. This is the position of the branch on the tree, which is different as each internode is added. This factor can be eliminated by using the new type of sequence that we have introduced (type 4), and when this is done configuration in the branches is found to be essentially like that in the main axis and is under the influence of extrinsic factors. Since successive whorls of branches exist in different environments the configurations differ according to the contemporary position of the whorl on the tree when a ring is laid down. In the configurations of successive "contemporary" whorls the suppression of lower branches is again manifest.

The dependence of configuration on external factors has been revealed by a deliberate change in the environment of one tree. In interpreting the effects of such a change it must be kept in mind that all parts of the tree do not undergo a similar change in environment when the tree is liberated. The uppermost whorls of branches are well exposed both before and after the event; the lowermost whorls are heavily shaded both before and after. The intermediate whorls are the ones that benefit from release by changing from a condition of shading to one of good exposure. The response in terms of radial growth in both branches and main axis can be understood if these circumstances are kept in mind, and if one remembers that any internode of the main axis

is dependent on those branches that occur above it.

The value of including the primary branches in an analysis of the tree's growth is patent. It derives from the circumstance that each branch during its development automatically advances through a series of progressively inferior positions in the tree, and that the effects can be traced in successive annual rings in its successive internodes. Hence the series of branches provides information of great potential value in diagnosing the biological controls of growth.

Acknowledgments

The authors acknowledge with gratitude the support afforded by the Committee on Extra Mural Research Projects of the Department of Agriculture during preparation of this paper. The work on which it is based is an integral part of the programme established by the late Professor G. H. Duff for research on the growth of trees, and we are indebted to all who assisted in that programme. The late Professor B. W. Kelley of the University of Waterloo was in charge of the field work involved, including felling and sectioning of the trees used for analysis. We should like to thank Mr. Mark Holst of the Petawawa Forest Experiment Station. For the privilege of establishing experimental plots in plantations that yielded specimen trees we have to thank the Ontario Department of Lands and Forests and the Department of National Defence.

References

1. DUFF, G. H. and NOLAN, N. J. 1953. Growth and morphogenesis in the Canadian forest species. I. The controls of cambial and apical activity in *Pinus resinosa* Ait. Can. J. Botany, **31**, 471–513.
2. DUFF, G. H. and NOLAN, N. J. 1957. Growth and morphogenesis in the Canadian forest species. II. Specific increments and their relation to the quantity and activity of growth in *Pinus resinosa* Ait. Can. J. Botany, **35**, 527–572.
3. DUFF, G. H. and NOLAN, N. J. 1958. Growth and morphogenesis in the Canadian forest species. III. The time scale of morphogenesis at the stem apex of *Pinus resinosa* Ait. Can. J. Botany, **36**, 687–706.
4. ENGLE, L. G. and SMITH, N. F. 1950. Red pine growth ten years after thinning. Papers Mich. Acad. Sci. **36**, 101–109.
5. EVERSOLE, K. R. 1955. Spacing tests in a Douglas fir plantation. Forest Sci. **1**, 14–18.
6. GUILLEBAUD, W. H. 1951. Norway spruce spacing plots at Wessling, Bavaria. Forestry, **24**, 121–126.
7. HOLMSGAARD, E. 1955. Årringsanalyser af Danske skovtraeer (Tree-ring analysis of Danish forest trees. English summary). Det forstlige Forsogsvoesen i Danmark, **22**, 5–247.
8. MOTT, D. R., NAIRN, L. D., and COOK, J. A. 1957. Radial growth in forest trees and effects of insect defoliation. Forest Sci. **3**, 287–304.
9. ONAKA, F. 1950. The longitudinal distribution of radial increments in trees. Kyoto Univ. Forests Bull. **18**, 1–54.

GROWTH AND MORPHOGENESIS IN THE CANADIAN FOREST SPECIES

V. FURTHER STUDIES OF WOOD GROWTH IN BRANCHES AND MAIN AXIS OF PINUS RESINOSA AIT. UNDER CONDITIONS OF OPEN GROWTH, SUPPRESSION, AND RELEASE[1]

DOROTHY F. FORWARD[2] AND NORAH J. NOLAN[2]

Abstract

The analysis of growth in trunk and branches of four red pine trees of different situations is extended to include internode length, circumference, annual wood volume increment, and cambial area. The effects of position of a branch on the tree and of suppression and release are examined in reference to these growth indexes.

Increase in circumference responds in much the same way as radial growth, and branch position is important. The greatest annual increase in girth of any branch internode occurs when it is near the periphery and near the top of the tree.

Terminal growth of branches is less affected than radial or tangential growth by branch position if the tree is in the open but is suppressed by surrounding trees. Upon release of a tree only those axes whose local environment was actually improved showed an increase in apical growth.

The interpretation of annual wood volume increment is complicated by the fact that it is determined not only by internal and external conditions during the current growing season, but also by past growth, both apical and cambial. It is subject to the effects of suppression and of branch position, chiefly through the influence of these factors on the cambial growth component.

I. Introduction

The total annual product of wood growth in any internode of a tree may be expressed as the increment of wood volume. Biologically it is the product of cambial growth and apical growth. But the contributions made by these two meristems are separated in time. Not only is the duration of the seasonal growth period different for the two meristems but, except in a terminal internode, the longitudinal component was determined in a previous year when the internode was formed, while it is the cambial growth of the current year that contributes to the annual increment in wood volume. Cambial growth itself has two components, additive growth, which accounts for radial additions to the wood, and multiplicative growth, which increases the area of the cambium itself.

The numerical indices that describe the linear components of the growth of an internodal annual ring of wood are ring width, internode length, and mean circumference while the whole product is the increment of wood volume. Increment of cambial area is a self-explanatory term. Of these indices, the annual radial increment, or ring width, in the trunk and primary branches

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Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. The data reported were collected and analyzed according to a plan designed by the late Professor G. H. Duff, and are an integral part of the experimental investigation of growth of the pine tree that he established. The senior author, however, accepts responsibility for the material introduced and the views expressed in the present paper.

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of four specimens of red pine has been analyzed in the preceding paper of this series (5). The present paper extends the analysis to include the remaining indices of growth of the same four trees. *Specific* growth of cambial area and its relation to *specific* increment of volume will be examined separately in the following paper.

II. Materials and Derivation of Data

II.1. Specimen Trees and Preparation of Wood Sections

The data presented here are derived from the same four young trees whose ring widths formed the subject of the preceding paper, where the trees are described (5).

To recapitulate briefly, they were:

Tree K55.1—open grown in the Petawawa Forest Experiment Station, Chalk River, Ontario. Collected in 1955.

Tree C53.5—open grown in Camp Borden, Ontario. Collected in 1953.

Tree XA57.18—in a 6′×6′ plantation near Craighurst, Ontario. Collected in 1957.

Tree XA58.9—originally part of the same 6′×6′ plantation as XA57.18, but released in September, 1950, by thinning to a 24″×24″ spacing. Collected in 1958.

The felling of trees and preparation of wood sections was described in the preceding paper (5).

II.2. Measurement of Growth Indices and Derivation of Data

The measurement of internode length was direct, with the exception that a correction in length of the basal internode of each branch was made by estimation to allow for the portion buried in the trunk.

The "mean cambial circumference" $\pi\ (r_n+r_{n-1})$ is the arithmetic mean of the circumference of the cambium at the beginning and end of the formation of each annual ring, and could be estimated from the lengths of the internal and external radii of the ring (r_{n-1} and r_n respectively), the circumference of a circle being $2\pi r$. The "terminal circumference" $(2\pi r_n)$ of a ring is its final or external circumference. The terminal circumference of the outermost wood ring is the ultimate circumference of the woody portion of any internode. Wood volume increment $[\pi L(r_n-r_{n-1})\ (r_n + r_{n-1}$ where L is internode length, r_{n-1} and r_n the internal and external radii of a ring] is the algebraic product of internode length, ring width, and mean circumference. Mean cambial area $[\pi L\ (r_n + r_{n-1})]$ is the product of internode length and mean cambial circumference.

For analysis of growth of the trunk and branches the same three types of longitudinal sequence are employed as in the previous paper. Type 1 is a diagonal, or oblique, sequence including rings laid down in any one year in consecutive internodes proceeding away from the apex of the trunk or branch. Type 3 is a vertical sequence including internodal rings laid down by cambia of uniform age, and type 4 is a modified type 3 sequence, used in analyzing branch growth, that eliminates the effect of changing branch position on the tree as it develops, and brings together internodes from branches having the

same serial position on the tree when the internodes were formed. A more complete description of these various types of sequences may be found in earlier papers (1, 5).

The concepts of "pattern" and "configuration" are used in the same sense as in earlier papers of the series (1, 2, 5). As in discussing ring width, configuration in branches may refer to type 3 or type 4 sequences.

III. Terminal Growth

The first component of growth to be examined in this paper is internode length which, in our four trees, represents the annual addition to length of a stem or branch.

Since the primary meristem is always at the apex of its axis and produces only one internode per season, the length of the internode may be expected to show the characteristics of *configuration* but not of *pattern*.

III.1. Terminal Growth of Main Axis

It was revealed in an earlier analysis (1) that the trend in terminal growth of the main axis conforms to that of the configuration of cambial growth. That this is also true of the four trees now under examination may be confirmed by comparison of the sequences of internode length in their main axes, which appear as solid lines in Fig. 1, with the type 3 cambial growth sequences for main axes which appear in the previous paper (5, Figs. 7–10). It is also true that annual fluctuations present a generally similar picture, and that instances of *precession of the cambium* (1) may be discerned. For example, in the open grown tree C53.5 (5, Fig. 8) peak values of ring width appeared in 1942 and 1945, and corresponding peak values of internode length (Fig. 1) in 1943 and 1946. This is selected as a conspicuous example, but others are also to be found on close inspection of the graphs. As already explained (1), it is not to be expected that this would be an invariable relationship, but its appearance indicates that in the trees with which we are concerned, as in those analyzed earlier, there is evidence that conditions existing at the time of formation of the terminal bud may frequently have a prominent effect on the internode length achieved in the following year.

In trees XA57.18 and XA58.9, which were sister trees in a 6′×6′ plantation until 1950, there is close correspondence in trend of internode length from 1940–1950, ending with a definite drop from 1949 to 1950. This follows upon a sharp drop in radial growth in both trees from 1948 to 1949, and provides another instance of precession of the cambium. But the released tree XA58.9 shows a departure from the general rule of correspondence of cambial and terminal growth. This appears as a marked depression of internode length after liberation of the tree at the end of 1950, and lasts until 1954 when internode length regains the approximate magnitude of 1948–1950. During the same period of time, from 1951 to 1953, ring width was greatly increased (cf. 5, Fig. 10). In the meantime the companion tree XA57.18, which remained in a 6′×6′ stand, showed mild fluctuations but no essential change in internode length. Such a response to release of a tree is unexpected. Hummel (8) reported average heights of two species of pine that were the same in thinned

and unthinned plots, and Guillebaud (7) found no difference in height of Norway spruce grown at spacings varying from about 4 ft to 13 ft. Eversole (4) reported that Douglas fir trees were taller at 10 or 12 ft spacing than at 4 to 8 ft. Engle and Smith (3) found no increase in height of red pine when a 5′×5′ plantation was thinned but a significant increase when a denser natural stand was thinned. A reduction in height after liberation is unexpected. It is therefore a matter of interest to know whether the temporary depression of terminal growth in tree XA58.9 is fortuitous or is actually a consequence of removal of its neighbors. Fortunately the internode length in the main

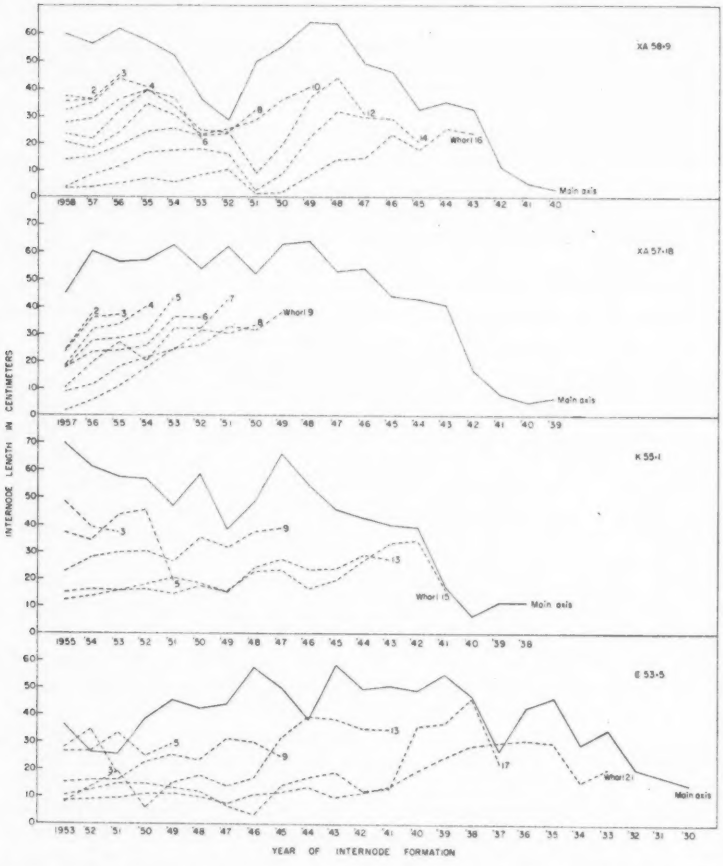

FIG. 1. Internode lengths of main axis and branches of four trees. Solid lines represent the main axis, broken lines the branches. The number subtending each sequence is the number of the whorl in which the branch occurred when the tree was cut.

axis of a number of trees in the Craighurst plantation have been measured, and we may compare suppressed and released trees in three situations within the plantation, which was subdivided into three areas of which XA was on a southern slope, XB on level ground at the bottom of the hill, and XC on level ground at the top.

Figure 2 shows internode lengths of 29 trees in all, 17 of which were released while 12 remained suppressed. Figure 2C represents four released and four suppressed trees from the upper level. Lines represent individual trees and crosses show the average for the group in each year. A vertical line marks the time of release of trees in the group marked R, while those marked S remained suppressed. The two sets of sequences correspond well, and no obvious effect of release on internode length is to be seen in this location. There is an increase in 1951 over 1950, but this is similar in both suppressed and released trees and reflects poor conditions at the site in 1949–1950. Figure 2B compares trees in the lower level of the plantation. All of the suppressed and one of the released trees show a fairly steady upward drift from 1951 to 1957. Two released trees show a small temporary depression in 1952–1953, while only one shows a major decrease extending from 1952 to 1954. Figure 2A represents four suppressed and nine released trees on the hillside,

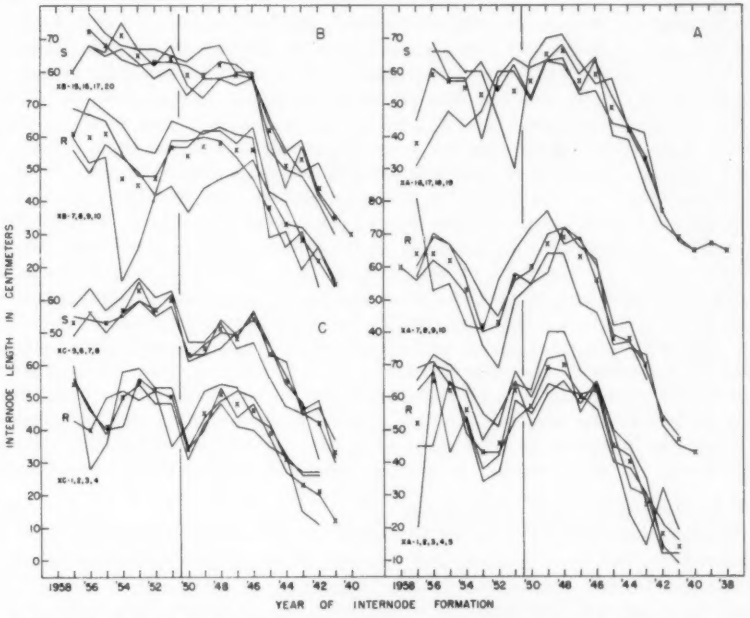

FIG. 2. Internode lengths of main axis of trees in the Craighurst plantation; S, suppressed, R, trees released in September 1950. Lines represent individual trees, crosses the yearly average for a group. A—trees on the hillside; B—on the lower level; C—on the upper level.

and here all nine released trees show a major dip in internode length in 1952–1953 or 1952–1954. This was of greatest magnitude in XA58.9, the tree chosen for complete analysis and represented in Fig. 1. Of the four suppressed trees one was uniform in internode length with only mild fluctuations between 1951 and 1956. This was XA57.18, also shown in Fig. 1. The contrast between these two trees therefore shows the maximum deviation of a released from a suppressed tree. The other three suppressed trees on the slope had at least one very short internode in the years after 1950, one being of minimum length in 1951, one in 1953 and one in 1957. A single tree may therefore produce a short internode for 1 or 2 years while sister trees grow normally, and this may happen without reference to the release of the tree. However, since the nine released trees on the hillside all had depressed longitudinal growth in the years 1952–1953 with partial recovery in 1954, this must be recognized as a consistent feature of the configuration of terminal growth of released trees in this particular situation. That it is related to the situation is obvious because only one of four trees on the lower levels showed a depression of similar magnitude in the same years, and none of the four on the upper level.

While it therefore appears that a temporary depression of longitudinal growth of trees on the hillside is a consequence of thinning the plot, it is by no means easy to account for it. It is true that XA58.9 produced a few cones in the years of minimum internode length, while XA57.18 did not. In 1952, for instance, XA58.9 had nine female cones at the peak of the tree (first two whorls) of which two were at the base of the leader and in 1953 it had six cones on the leader and the first whorl of branches. It had none in 1954 or 1955 and the suppressed tree XA57.18 bore no cones in any of these years. But it is doubtful whether such sparse cone production can be held responsible for a drastic reduction in terminal growth of the leader. The years 1949 to 1952 were four consecutive years of low rainfall in May and June in the area, and as a consequence one might expect poor terminal growth in 1950–1953. If water stress were the limiting factor for growth, then exposed trees on the hillside might be expected to feel it more than closely planted trees which would transpire less, or trees on level ground where surface runoff would be less. Whatever the explanation the depression was temporary and ultimately the released trees produced internodes comparable in length to those formed before their release. The final effect of release of the tree, then, is to cause no significant change in the annual terminal growth of the main axis. Since this occurs at the apex of the tree, whose environment is not affected by release, the outcome is consistent with the hypothesis advanced by Duff and Nolan (1) that terminal growth is under the control of extrinsic factors.

III.2. Terminal Growth of Branches

We have seen that the cambial growth of branches is affected by the external situation of the tree as a whole and also by the situation within the tree of the individual branch concerned. It may therefore be supposed, if the general analysis is correct, that terminal growth will be similarly affected. That it is will be readily observed by reference to Fig. 1 where the internode lengths of branches are shown as broken lines, numbered according to the whorl in which each branch occurred. Since each internode of a branch is formed by growth of

the terminal bud of that branch, each internode achieves its full length while in a peripheral position on the tree. Consequently one would expect the suppressing effect of overlying branches to be minimal in open grown trees, and maximal in a tree closely surrounded and shaded by its neighbors. This is manifest in Fig. 1. In the open grown trees K55.1 and C53.5 the branches from whorls below the top five show a consistent, though slight, annual decline in internode length (as in ring width, cf. 5, Figs. 7–10), whether the main axis shows an upward, uniform, or downward trend. But the downward trend in the branches of these trees is mild compared with the strong downward trend displayed by the branches of the close plantation tree XA57.18. That this sharpening of the suppression of terminal growth of the branches is the result of interference on the part of closely neighboring trees becomes clear when we examine the internode lengths of branches in tree XA58.9. Until 1950 this tree was similar in situation to tree XA57.18 and the branches show a similar decline in terminal growth in whorls 12–16. The only exception is whorl 10, which, in 1950, was the second whorl on the tree and therefore not greatly affected by the one young whorl above it. It will be noticed that the internode developed in 1951 from the 1950 bud was still shorter than that developed in 1950, which provides a conspicuous example of the effect of conditions during development of the bud upon longitudinal growth in the following year. At the end of 1950 the near neighbors were removed from the vicinity of this tree, and the buds formed in 1951 produced markedly longer internodes in 1952 in the lower whorls but not in those which were then near the top of the tree. This can only be interpreted as the effect of releasing the tips of the lower branches from suppression by neighboring trees, especially since thereafter the decline in successive years was much less steep, and much more nearly like the picture presented by the open grown trees than that presented by tree XA57.18, which remained suppressed during the period 1950–1957.

The internode lengths of branches as recorded in Fig. 1 bear out, in a general way, the observation of Friesner (6) that in any one year the terminal growth of lower branches is less than that of higher branches, and, with few exceptions, the terminal growth of upper branches is less than that of the primary axis.

We have seen earlier (5) that configuration of ring width in a branch is affected by the circumstance that successive internodes from apex to base of

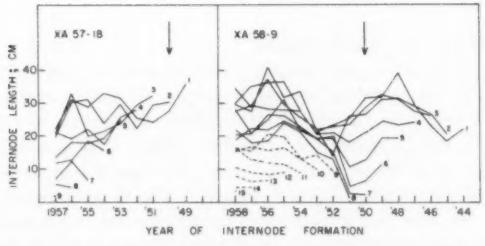

FIG. 3. Type 4 sequences of internode length in the branches of trees XA57.18 and XA58.9. The number subtending each sequence is the number of the whorl in which the internodes were initiated. For further explanation see text.

the branch were formed when the branch was in a higher and higher relative position on the tree, and that this effect can be eliminated by using a type 4 sequence in which the "contemporary" serial number of a whorl is used. Consequently type 4 sequences were compiled for internode length of branches of tree XA58.9 and XA57.18, and are shown in Fig. 3. These sequences were derived in the same manner as type 4 sequences of ring width, and a detailed account of their derivation is given in the previous paper (5). This type of sequence eliminates the effect of changing relative position of the branch as it develops and portrays the succession of internodes each of which was contemporarily in the position shown by the whorl number in Fig. 3 when the internode in question was formed. Each internode length appearing in Fig. 3 is an average for four to eight internodes in different branches of the same whorl.

In tree XA57.18, only in the top three whorls did internodes grow to a length of 20–30 cm in all years from 1950–1957. Below the third whorl, successively lower whorls had formed shorter internodes during the same years in this close-plantation tree.

Tree XA58.9 gives a similar picture in the years before its release, 1944–1950. After 1950, however, when neighboring trees were removed, internodes formed in whorls as low as the 8th had, in later years, lengths above 20 cm, and only those formed in the 9th and lower whorls showed progressive intra-tree suppression. The sequences for these lower whorls appear as broken lines in Fig. 3. Whorls as low as the 11th were able to respond in some measure to release of the tree by improved terminal growth in subsequent years. The configuration of whorls 10 and 11 was virtually free from the random effects of annual variation in external conditions, which suggests that here the exposure of the periphery of the tree to better illumination was an overriding factor in terminal growth of branches in this position. In still lower whorls no improvement occurred, perhaps because by the time they were in this position encroachment of neighboring trees as well as the improved growth of overlying branches brought about suppression of even the apical portions of these branches.

In the upper whorls of tree XA58.9 there is in the branches as well as in the main axis (Figs. 1, 2), a tendency to temporary depression of terminal growth in the years 1952–1953 that is not duplicated in the suppressed tree XA57.18; but from the 7th to the 11th whorl improved exposure was the dominant influence.

III.3. Discussion of Terminal Growth

The sequences of internode length in the main axis have the attributes of *configuration* since the growth in length occurs always in the apical internode. Sequences of internode length in branches, however, are affected by an intrinsic factor related to branch position. When this is eliminated by using type 4 sequences it is found that under conditions of suppression and release the sequences of internodes formed when in the three uppermost whorls are similar to that of the main axis, and after release, terminal growth never greatly exceeds that before release. But the sequences of lower whorls show a marked improvement in terminal growth after release, until one comes to those laid down in the lowermost whorls where no improvement occurs. The apices

of trunk and branches at the top of the tree were well exposed even in the 6′×6′ stand and underwent no effective change in environment when the plot was thinned. The apices of the lowermost branches were always heavily shaded and underwent no effective change in environment. But the apices of intermediate branches were shaded in the 6′×6′ stand, but freely exposed in the 24′×24′ stand, and it was in intermediate positions that the terminal internodes of branches achieved substantially greater growth in later years than in 1950 – 1951.

IV. Increase in Circumference

We next consider growth in circumference, which is the third linear component of the volume of an internodal annual ring of wood. This component derives from cambial growth which involves production of new cells by both periclinal and anticlinal divisions and, however complicated these processes may be, they result in both radial and tangential additions to the permanent tissue and increase the amount of the cambium itself. We employ as a unit the "mean cambial circumference", i.e. the arithmetic mean of the internal and the external circumference of a wood ring, which represents the mean circumference of the self-replicating layer of the cambium because this is at all times on the periphery of the wood ring.

IV.1. Growth in Circumference of Trunk and Branches

Families of type 1 sequences of mean cambial circumference of all rings in all internodes of the main axes of our four specimen trees have been plotted, but for reasons of economy of space they are not reproduced here. They are similar in form to such sequences illustrated by Duff and Nolan (2). The open grown tree C53.5 presented a picture much like that of (2, Fig. 14); the plantation trees XA57.18 and XA58.9 much like that of (2, Fig. 15). The family of sequences for K55.1, while having a higher pitch than that of any of the other trees, also showed a certain amount of tailing off of individual sequences as did the plantation trees.

A comparison of the maximum, or limiting, mean circumference reached in successive internodes of the main axes of all four trees is set forth in Fig. 4.

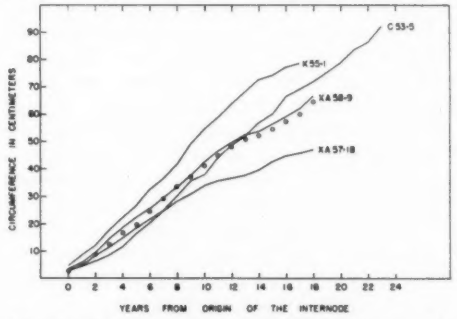

FIG. 4. Internodal sequences of maximum mean circumference for four trees, shown as solid lines. Internodal sequence of terminal circumference for tree XA58.9 shown as circles. For explanation see text.

A graph was obtained for each tree by joining points representing the largest mean circumference recorded in each internode, counted from the apex of the axis, at any time during its development. In accordance with expectations arising from an earlier comparison of three trees (2), the four curves of Fig. 4 reflect the site conditions of the trees they represent. Increase in the circumference attained proceeds in approximately linear and nearly parallel fashion through all but the lowermost internodes, with the exception of the suppressed tree XA57.18. Tree XA58.9 benefited by its release and attained a greater circumference throughout the length of the trunk because of additional growth in the eight years following its release, divergence from the suppressed tree being greatest in the lower internodes of the tree where the total amount of cambium was greatest.

The regularity of form in individual sequences of circumference in the families belonging to a single tree suggest an intrinsic control of multiplicative growth of the cambium, the differences associated with site being small so long as the tree was not suppressed. Suppression, which affects internal nutritional gradients, had a greater effect.

A further means of assessing the influence of suppression can be found in a comparison of individual branches within a tree. Such a comparison of the maximum circumference achieved by individual internodes of a few selected branches of two trees appears in Fig. 5. The upper group of curves represents

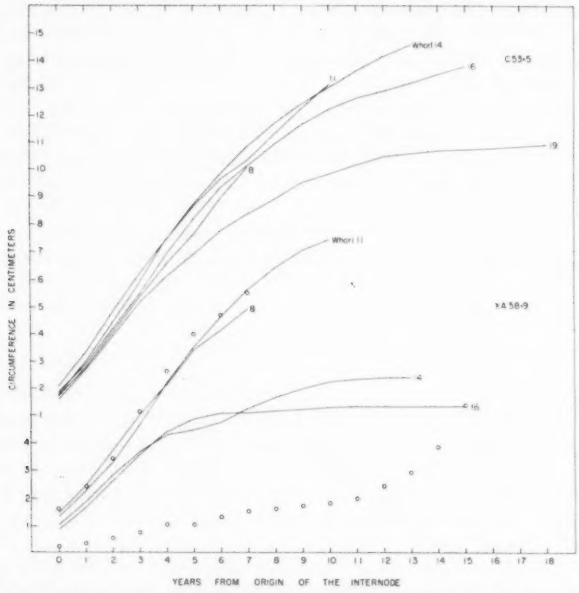

FIG. 5. Internodal sequences of maximum mean circumference for branches of trees C53.5 (upper) and XA58.9 (lower) shown as solid lines. Internodal sequences of terminal circumference for two branches shown as circles. Numbers subtending the sequences represent the number of the whorl in which the branch occurred when the tree was cut.

the maximum mean cambial circumference of five branches of the open grown tree C53.5. The number subtending each curve is that of the whorl in which the branch occurred when the tree was analyzed and the curve represents the maximum mean cambial circumference in successive internodes proceeding from the apex of the branch. In the branches of whorls 8 and 11 basipetal increase in circumference was approximately linear throughout, and this was true of branches in all of the uppermost 11 whorls. In branches of lower whorls represented by 16 and 19, increase was linear in the distal region, but tapered off in basal regions, and in whorl 19 a limit was reached in about the 13th internode beyond which the circumference increased only very slightly.

This phenomenon was much more conspicuous in tree XA58.9, which began its life in a close plantation. Branches from four whorls of this tree are represented in the lower group of curves in Fig. 5. Of these, the 8th whorl was initiated in 1951 after release of the tree and the growth in circumference was similar to that in whorl 8 of the open grown tree C53.5. Whorl 11 was the 3rd whorl of the tree at the time of its release, and only the basal three internodes of its branches had grown under conditions of suppression. Whorls 14 and 16 were, respectively, 7th and 9th from the top of the tree when it was released, and it is evident that they were already in so unfavorable a position as to suffer in subsequent years from suppression by the whorls above them. The branches of these whorls were in fact suppressed throughout their development, for when they were in a more favorable position on the tree, the tree itself was in a $6' \times 6'$ stand. This will account for the greater restriction evident in corresponding whorls in the lower regions of this tree as compared with those of tree C53.5, which showed the same tendency to suppression of lower branches by upper, but which achieved greater girth because of the contributions made in the early years when they were in a favorable position on a tree in an open situation.

A clear indication that branches in the lower whorls have a different distribution of circumferential growth may be obtained by further inspection of records for tree XA58.9 in Figs. 4 and 5. In addition to the maximum mean cambial circumference achieved, for example by the internode that was 1st, 2nd, 3rd, etc. from the apex of the branch at any stage in its development, we have plotted as circles the terminal circumference or ultimate perimeter of the wood cylinder in the successive internodes of two fully grown branches and the main axis as they existed when the tree was felled. In the main axis and in the branch from whorl 8 there was a linear increase in terminal circumference from apex to base, following very closely the drift of maximal mean circumference. But in whorl 16 the picture is very different. Here the final girth of all but the basal internode of the fully grown branch falls well below that of internodes that were as far from the apex when the branch was shorter. For example, the maximum mean circumference of any 5th internode of this branch was between 4 and 5 cm and was achieved in the basal one of five internodes when the branch was in the 5th whorl. That same internode remained, of course, the basal one and is now the 16th, but it increased only slightly more in girth. The 5th internode in the branch when it was in whorl 16 (terminal circumference) was only about 1 cm in girth, and only a few basal internodes finally attained a girth of more than 2 cm. The greater

ultimate circumference attained in the four or five basal internodes of whorl 16 resulted from good growth of their inner rings while the branch was among the upper four or five whorls of the tree.

It has been suggested (2) that the sequence of internodal perimeters along an axis will serve as an approximate index to the activity of multiplicative cambial growth. The present data confirm this so far as the main axis and upper branches are concerned, but it would be quite inadequate for lower branches.

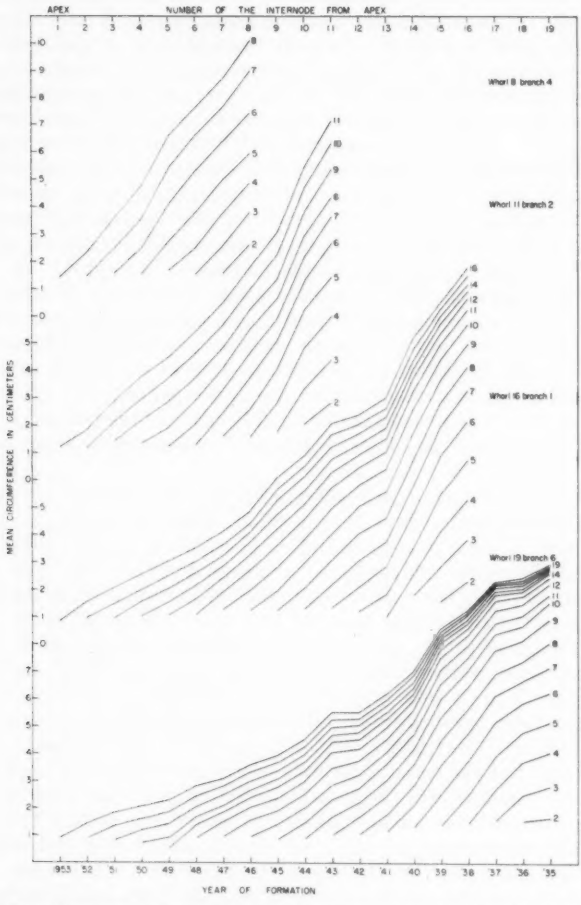

FIG. 6. Type 1 sequence of mean cambial circumference for four branches of tree C53.5, plotted in the first mode, with the origin of each sequence appropriately placed on a time axis. Each sequence is numbered to show the number of the whorl in which the branch occurred when the ring was laid down.

The growth in circumference of the branches that we have been considering is more fully portrayed in Figs. 6 and 7, where mean cambial circumference of their individual rings is shown. In Fig. 6 are all the type 1 sequences for four branches of the open grown tree C53.5, plotted in the first mode so that chronology is preserved and all rings of a single internode of the fully developed branch lie in a vertical series. The branch from whorl 8 represents the branches of all higher whorls as well, while those from whorls 11, 14, and 19 represent the changes that extend to the lowest whorl, number 22, which was much like whorl 19.

In Fig. 6 type 1 sequences show, within each sequence, mean circumferences of all internodal rings from apex to base laid down in a single year, while the separate sequences, proceeding from the lower right hand corner, represent successive annual rings from the pith outward. The figure subtending each sequence is the number of the whorl in which the branch occurred when the ring was laid down. In whorl 8, the linear form of sequences and the relatively uniform intersequential spacing indicates that the circumference increased more or less uniformly throughout the growth of this branch. The branch from whorl 11 differs in the upper right hand portion, where circumference increased less in the basal internodes after the branch came to be in the 7th or 8th whorl. In the branch from the 16th whorl this suppression of later growth is more evident in the closer intersequential spacing in basal internodes of branches when they were in relatively low positions on the tree. Linearity is lost because of the steeper gradient in circumference imposed during the first few years of growth of the branch, and suppression extends to the distal internodes. The same features are accentuated in the branch from whorl 19.

It is apparent that the major increase in circumference of a lower branch occurs in the first 8 or 10 internodes formed in that branch. These internodes grew as the branch moved from the 1st to the 8th or 10th whorl, while the more distal ones, which increased by a smaller amount annually, grew while the branch was in a still lower whorl. Suppression of circumferential growth therefore corresponds to suppression of ring width which was evident in this open-grown tree only in whorls lower than the 8th (5).

Tree XA58.9—released from suppression: the data for this tree are in Fig. 7. Here the same relationships are patent, differing only quantitatively from those of Fig. 6. All rings of the 8th whorl and all but the three innermost rings of the whorl 11 branches developed after release of the tree in 1950. The array of sequences in the branch from whorl 8 is closely similar to that of the 8th whorl of tree C53.5 shown in Fig. 6. The whorl 11 sequences are also typical of a branch advancing from the 3rd to the 11th whorl in a tree growing in open conditions. It shows suppression in the later formed rings undoubtedly because of severe shading by the whorls of branches that ultimately overlaid it. This branch shows no sign of suppression in the years before release because it had not yet advanced beyond the 3rd whorl. The branch from whorl 14, on the other hand, was in the 6th whorl in 1950, and was already showing suppression. This corresponds to our earlier observation (5) that ring width was restricted in all whorls below the 4th or 5th in a suppressed tree. Release of the tree at the end of 1950 opened up the surroundings and the rings of the immediately succeeding years were correspondingly greater in circumference.

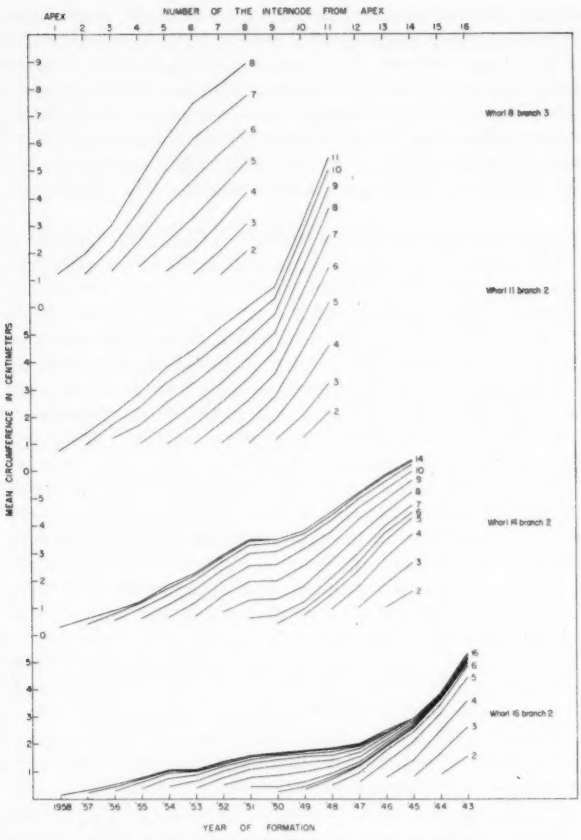

FIG. 7. Type 1 sequences of mean cambial circumference for four branches of the tree XA 58.9. For explanation see subscript to Fig. 6.

But the branch was soon overtopped by more than eight whorls and even in an open-grown tree a branch begins to show signs of suppression when this occurs. The final branch of this tree that is portrayed was in the 16th whorl in 1958, in the 8th in 1950. As in the preceding branch, suppression was evident in all whorls below the 5th while the tree remained in a close stand. After the neighboring trees were removed this branch already had eight whorls above it and so was unable to share in the improvement in exposure. This branch may be contrasted with that of whorl 16 in tree C53.5 (Fig. 6), which suffered no suppression until it was overshadowed by later-formed whorls of the tree itself. The only major increase in girth of the whorl 16 branch of tree XA58.9 occurred when the branch was less than 5 years old and in the top of the tree. Only in the basal internodes, therefore, was a substantial girth attained.

IV.2. Discussion of Growth in Circumference

This mode of analysis reveals that the girth attained in any year by any internodal cambium in a branch is determined by an intrinsic factor or factors bearing a definite relationship to displacement from the base of the branch. Since each internode was at the periphery of the tree when it was formed, and the number of the internode as counted from the base of the branch corresponds to the position of the whorl in which it was initiated, it becomes clear that the major increase in girth of any branch internode occurred when it was both near the periphery and near the top of the tree. This is the same position that, as we have seen, favors maximum radial increment, or ring width. Increase in girth results from multiplicative growth of the cambium, but this is not independent of additive growth, or wood formation, for, as more wood is laid down, the cambium, which constantly occupies the periphery of the currently growing wood ring, must increase proportionally. The control of the two kinds of cambial growth will come under discussion in the following paper, and here we merely record the observation that as a branch grows and simultaneously changes its relative position on the tree its circumferential growth responds in the same way as does its radial growth.

V. Wood Volume Increment

In the preceding paper and in the foregoing sections of the present paper we have examined the three unidimensional components of growth of an internode: (1) vertical, resulting from longitudinal growth and represented by internode length; (2) tangential, resulting from multiplicative increase in cambial circumference; and (3) radial, resulting from additive growth on the part of the cambium and represented by ring width. Wood volume increment is the total annual production of wood by an internodal cambium and, geometrically, is the product of internode length, mean circumference, and width of the ring. The last of these components makes a numerically small contribution to the product, and we must regard the other two as the major numerical determinants of wood volume increment. However, the *relative* differences in ring width from one portion of a tree to another, or from one tree to another, may be as great as relative differences in circumference or internode length, so in spite of its numerically small magnitude, ring width is not negligible when we seek to compare production of wood volume in different internodes.

Biologically, ring width and circumference are both derivatives of cambial growth and may be considered together as one biological component of wood growth, the other being internode length which is the outcome of growth of the apical meristem. Internode length, once set, remains unchanged, so that, except in a terminal internode, the longitudinal component of the volume of wood produced in any one year depends on terminal growth determined in previous years. The radial component, ring width, depends on cambial growth in the current season. The mean circumference depends on multiplicative growth of the cambium both in the current year and in past years, and the relative contributions of past and present will vary with the position of the internodal ring. The distribution of annual wood production in a tree is therefore a complex matter. Since the growth of the apical meristem appears to be

FIG. 8. Type 1 sequence of wood volume increment and its linear components. A—tree K55.1; B—tree XA58.9; C—tree XA57.18. In the upper section the width of selected annual rings appears as solid lines, their mean circumference as circles, and their internode length as lines with crosses. In the lower section solid lines show the pattern of wood volume increment in the main axis, broken lines in branches.

under the control of extrinsic factors and that of the cambium primarily under the influence of intrinsic factors (1, 2, 5) the increment of wood volume is affected by both. We propose to describe in this section the annual increment of wood volume in trunk and branches of the four comparison trees and to consider the factors determining its distribution in the trees.

V.1. Wood Volume Increment in the Main Axis

In Fig. 8 are data for three trees showing increment of wood volume and its linear components. Fig. 8A represents the open grown tree K55.1. In the lower part of the figure are graphs of wood volume increment for two annual rings of the main axis, which were laid down in 1955 and in 1949. They appear as solid lines. Broken lines show, on a different scale, wood volume increment in the 1955 ring of branches, and these will be referred to later. In the upper part of the figure are the linear components of wood volume for the main axis, which we shall consider first. It will be noticed that ring width is plotted on a scale 20 times that used for internode length and circumference of the ring. Figs. 8B and 8C contain similar data for tree XA58.9, released from suppression, and for XA57.18, the close plantation tree.

The graphs showing wood volume are type 1 sequences. As always they proceed down the tree, internode by internode, from the apex at the left towards the base as one moves to the right. The pattern of annual increment of wood volume consists of a more or less regular steep rise to a maximum, soon followed by a sharp decline which is less regular in form, but exhibits annual fluctuations which correspond to those of internode length and are obviously imposed by this component.

The determinants of distribution of wood volume increment within a tree have been discussed by Duff and Nolan (2) and the present data are examined in the light of their interpretation. In a type 1 sequence the circumference of an internodal ring increases progressively from the apex of the axis downwards, the ring width increases sharply for a few internodes and then declines more or less, while in the three trees here under consideration the internode length undergoes sharp fluctuations but maintains an over-all uniformity (or in K55.1 a mild decline) in the upper 10 or 12 internodes, below which it falls away steeply. The basipetal increase in annual wood production proceeding from the apex of the tree conforms to the regular increase in circumference and extends far beyond the initial increase in ring width. The fluctuations exhibited by internode length are absent from the early portion of a sequence of wood volume increment, but gradually they increase in magnitude, and beyond the position of the maximum in the sequence they correspond closely to those of internode length. It is clear that, in the basipetally rising phase of wood volume increment, increasing circumference is at first the dominant determinant, but that there is a gradually augmenting participation of internode length, and during the falling phase of volume increment the latter is the dominant component. A type 1 sequence passes through rings that in upper internodes lie close to the pith but in lower ones surround an increasing number of rings laid down in former years. The increase in circumference is, of course, the outcome of total cambial growth but more and more of it pertains to cambial growth of former seasons as one

tree XA57.18. In the upper section the width of selected annual rings appears as solid lines, their mean circumference as circles, and their internode length as lines with crosses. In the lower section solid lines show the pattern of wood volume increment in the main axis, broken lines in branches.

progresses down the axis. Although the absolute increment of circumference in consecutive internodes is fairly uniform the relative increment diminishes. In the first few internodes the annual increment in mean circumference is more than 50%. At the end of the phase where circumference dominates wood volume it has fallen to about 20%, and in the lowest internodes examined it is 5% or less. It is therefore where circumference is changing by less than 20% per year that internode length exerts its full influence on wood volume.

The foregoing considerations would apply to any one of the rings shown in Fig. 8 for the three trees although there are obvious quantitative differences among trees. In the first place, it is clear that the open-grown tree K55.1 was the most productive of wood in a given ring, while the suppressed tree XA57.18 was least productive. Inspection of the upper sections of the figure will show that differences in internode length were not conspicuous, and that the products of cambial growth (ring width and circumference) were chiefly responsible for the large differences among trees in wood production.

Another conspicuous difference between trees lies in the number of internodes in the rising phase of the sequences, and therefore in the phase where wood volume is determined by total cambial growth. In K55.1, grown in the open, nine internodes of the last-formed ring lie in this phase; in XA58.9, released in 1950, 10 internodes; but in XA57.18, the suppressed tree, only five internodes comprise the steeply rising phase.

The second open grown tree, C53.5, exhibited similar features, as may be seen in Fig. 9 where the abscissal scale is more extended. Here the wood volume increment in two annual rings of the main axis is again shown by solid lines, together with internode length. Ring width and mean circumference are omitted, but they were similar in pattern to those of the other three trees. The strong influence of internode length on wood volume increment below the 10th or 11th internode is very conspicuous here, while the failure of wood production to correspond to internode length in the upper portion of the tree reveals the controlling influence of total cambial growth in this region.

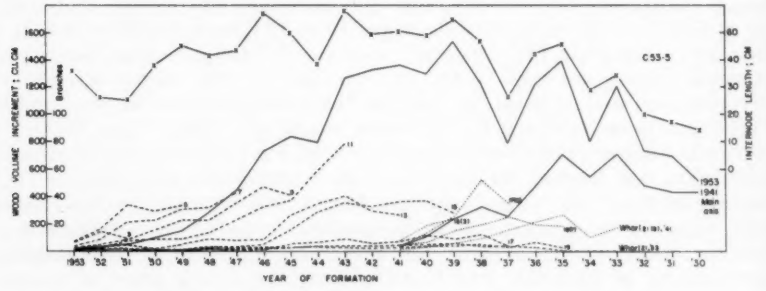

FIG. 9. Type 1 sequences of wood volume increment in tree C53.5. The line with crosses represents internode length. Solid lines represent sequences of volume in two annual rings of the main axis. Broken lines show volumes in the rings laid down in 1953 by branches in different whorls. Dotted lines show volumes in rings laid down in 1941 by branches. The numbers subtending branch sequences are the numbers of the whorls in which the branches occurred in 1953 (those in parentheses the whorls in which they occurred in 1941).

These observations on the pattern of wood production in the main axis of our four trees conform to those on earlier trees and serve to confirm the principles derived from them by Duff and Nolan (2). The maximating and then declining pattern of wood volume increment can be construed as a transition from the dominant effect of total cambial growth, in the upper part of the tree to the dominant effect of past apical growth in the lower part. Control by the cambium spreads farther down the tree in open conditions where foliated branches are better exposed to light and consequently provide for a greater access of nutrients to the main axis at their bases, for here the total amount of cambium increases at a high relative rate through more internodes.

That the effective factor is total amount of growing cambium may be seen by comparing the pattern of wood volume increment in tree XA58.9 (Fig. 8B) with that of mean cambial area (Fig. 11). The latter is, of course, the product of mean circumference and internode length. The third component of volume, ring width, being numerically small, makes a modest contribution to volume but does not change the general form of its pattern.

V.2. Wood Volume Increment in the Branches

The production of wood in the branches is similar in pattern to that in the main axis. Broken lines in Fig. 8 represent sequences of wood volume increment extending back from the apices of various branches during the last year of growth of each tree. The number subtending each branch sequence is that of the whorl in which the branch occurred. In tree K55.1 (Fig. 8A) wood volume increment, although absolutely less than in the main axis, followed a similarly steep increase away from the apex of the branch in the top nine whorls, while in lower whorls wood production was suppressed. In tree XA58.9 (Fig. 8B) a steep increase occurred in the branches of the top 10 whorls, but not in lower ones. In XA57.18 (Fig. 8C), which was closely surrounded by other trees, secondary suppression of wood production was evident in branches below the top five whorls.

In Fig. 9 broken lines show wood volume in branch rings laid down in 1953 by tree C53.5. Again, in the uppermost 11 whorls, i.e. in that portion of the tree where wood production was increasing basipetally in the main axis, there was also an increase away from the tips of the branches, while branches of lower whorls were unable to keep pace in wood production. The branch rings laid down in 1941 are shown in Fig. 9 as dotted lines, and by comparing those laid down in 1953 in corresponding whorls, one can see the effects of suppression due to position on the tree. The first internode of the main axis sequence of 1941 was then at the apex of the tree, and its rising phase of wood volume increment was under control of cambial growth in what was then the upper portion of the tree. The 15th whorl of 1953 was in 1941 the 3rd, the 17th was the 5th, and so on. (The 1941 position of each whorl is shown in parentheses.) In 1953 the production of wood in a branch of the 17th whorl was negligible, but in 1941 when the same branch was in the 5th whorl it made an appreciable increment in wood volume. So also did the branch that was then in the 9th whorl, while this same branch in 1953 when it was in the 21st whorl was virtually incapable of wood production.

Reference to Fig. 11 will show that the secondary suppression of cambial

activity in branches of lower whorls results in small cambial area even in the outermost rings and, as in the main axis, this appears to be the effective determinant of wood volume increment. It depends, of course, on both internode length and mean circumference.

While the wood volume increment of branches has been included in Figs. 8 and 9, none of the linear components for branches are shown there, and in order to learn whether the same controls are exercised within an individual branch as in the main axis, it is necessary to plot at least the internode lengths for certain branches along with wood volume sequences. This is done in Fig. 10 for a selection of the last-formed branch rings from three trees. Solid lines represent internode length, broken lines wood volume. Because it is desirable to include several branches from each tree, the inclusion of all the linear components of volume would lead to confusion. It may be assumed that circumference increases steadily away from the apex of the branch and that ring width (much smaller in magnitude) increases for two to four internodes in the upper 9 or 10 whorls in an open-grown, or 5 whorls in the close plantation tree.

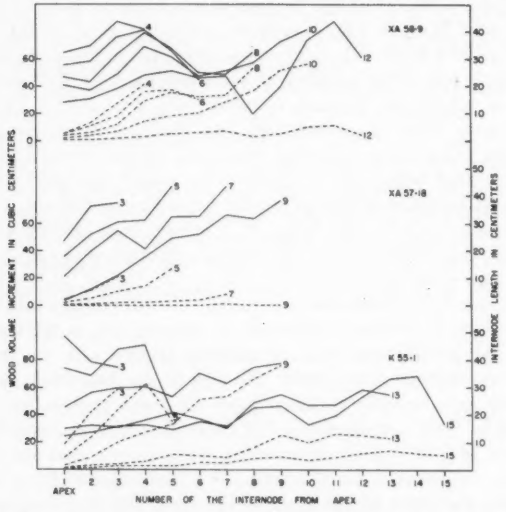

FIG. 10. Wood volume increment and internode length in branches of three trees. Solid lines represent internode lengths. Broken lines represent the type 1 sequences of wood volume increment in the final year of each tree's existence. The numbers subtending the sequences are whorl numbers.

Internodes in the branches are somewhat shorter than those of the main axis, of the general order of 20 to 80% according to position. But wood volume is of an altogether lower order of magnitude, a branch internode being slenderer than one in the trunk. Numerically, therefore, internode length is the largest component of wood volume in a branch and one might expect its trend to be

strongly imposed on the sequences of wood volume increment. In spite of this it is clear that in the open grown tree K55.1, shown in the lower portion of Fig. 10, wood volume increment fails to correspond to internode length in the distal parts of branches in the top nine whorls. Here, therefore, as in the upper portion of the main axis, wood production is limited by the components produced by the cambium. In the 13th whorl volume increment parallels internode length throughout the branch, but is small in amount, and in whorl 15 it is even more depressed although internode length is not essentially different from the sequence of whorl 13. In these lower whorls we see the effect of secondary suppression of cambial activity imposed by the overlying branches, while internode length, determined in successive years at the periphery of the tree, is relatively mildly suppressed.

Tree XA58.9, shown at the top of Fig. 10, was released in 1950 and by 1958 was again showing a mild degree of interference from surrounding trees. Under these conditions determination of wood increment by total cambial growth is evident in the top eight whorls of branches, for here wood volume rises as one proceeds away from the branch apex while internode length shows considerable annual variation but no steady drift. In the basal portion of a branch in whorl 8 wood volume parallels internode length and throughout whorl 10 the two correspond. But the 1958 ring laid down in whorl 12 produced very little wood while internode length underwent large changes within the sequence. Here, then, the secondary suppression of wood production by the cambium, both past and present, imposed by overlying whorls, extends throughout the branch and keeps the amount of growing cambium small (cf. Fig. 11, whorl 14, branch 2).

We have seen that in trees with good peripheral exposure the intratree suppression of terminal growth is mild. But in tree XA57.18, which was closely surrounded by other trees, internode length in the branches decreased sharply as a branch was overlaid by new whorls, and the rising sequence of internode length combines with a rising sequence of circumference to give a rising sequence of wood volume increment in the top five whorls. It is not possible in this tree to segregate the effects of length and circumference of the internode upon wood volume in the upper branches. In whorls below the 5th, however, the secondary suppression of wood growth by suppression of cambial activity becomes evident in the very small wood volume increments of whorls 7 and 9 even in internodes of lengths nearly equivalent to those of the 3rd and 5th whorls.

V.3. Discussion of Wood Volume Increment

In the total annual production of wood the four trees examined here bear out the principles evolved to explain the observations made on earlier trees (1), namely that in the upper portion of the main axis of a tree the production of wood in any year is dominated by the status of the cambium, while in the lower portion it is determined mainly by the past activity of the apical meristem, and that the extent of these two regions is related to the external situation of the tree. In an open-grown tree where foliage-bearing branches extend down a longer portion of the trunk, the influence of the cambium is extended.

The branches reflect the situation in the main axis so long as they are in an exposed position, but when they are overshadowed by higher whorls or by neighboring trees, a secondary suppression of cambial growth again limits the production of wood. Upper branches have few internodes all or most of which are included in the distal region where wood volume increment is limited by increase in circumference. Older, lower, branches have more internodes, and internode length might be expected to be the dominant component of volume increment in their basal portions. But secondary suppression of cambial activity again places wood production under limitation by total cambial growth. The result is that, in branches, internode length is a less important determinant of wood volume than in the main axis, and the comparative slenderness of a branch internode is dictated by either primary or primary and secondary limitation of cambial growth.

The foregoing analysis has been confined to sequences of type 1 because vertical sequences of the 3rd and 4th types such as were used to reveal configuration of radial increment would not have the same meaning if applied to wood volume increment. This is because neither of the chief components, internode length or mean circumference, depends solely on events of the current year. The length of an internode, fixed when it is initiated, affects the volume of all annual increments in that internode in subsequent years. The radius of the wood cylinder formed in preceding years influences the mean circumference of any ring. Only the numerically small radial component, ring width, is determined wholly within the current year. Consequently the volume of wood produced in an internode in any year is dependent on a complex of events, past and present, and vertical sequences will not align the effects upon cambia of uniform age of conditions within a single growing season. That is to say, the vertical sequence will not have the attributes of configuration.

The production of wood and the factors affecting it are important both from a practical and a theoretical point of view, but because temporal and spatial elements in its determination are inextricable, the distribution of wood volume increment cannot be used as a simple index to the operation of a particular factor. Nevertheless it has been possible to gain some insight into the principles underlying its distribution throughout the trunk and branches of a tree and to see at work the same factors that are more simply related to other indices of growth. It is clear that the same influences operate in branches as in the main axis and are modified by the position of the branch on the tree.

VI. Mean Cambial Area

A brief section on mean cambial area is included because it provides a measure of the amount of growing cambium in any internodal ring. It has already been shown that this is the prime determinant of volume increment, and it is a necessary datum for assessment of specific activity of the cambium which will receive attention in the following paper. Its co-ordinates are the length of the internode and the mean circumference, and since the former is the outcome of activity of the apical meristem and the latter of multiplicative growth of the cambium, the area achieved is the resultant of these two processes each

of which, as we have seen, had made some or all of its contribution in previous years. It will at once be obvious that mean cambial area differs from wood volume increment only in the exclusion of ring width as a component, and that most of the considerations concerning the distribution of the latter apply also to the former.

The pattern of mean cambial area is illustrated in Fig. 11 for one tree only. In Fig. 11 all annual rings for the main axis and two branches of tree XA58.9 are represented. By comparison with Fig. 8B it may be seen that the pattern is similar to that of wood volume increment, modified somewhat, but unchanged in essentials by omission of ring width. The variations in pattern of volume increment shown in Figs. 8 and 9 will therefore indicate the variation in pattern of cambial area in the trees that are not illustrated. Internode length is included in Fig. 11, and it should be observed that that of the main axis is on a displaced ordinate scale, and falls to very small values in the last internodes of the sequence. It is obvious that in the lower internodes of the main axis cambial area follows the trend and exhibits the fluctuations of internode length. In the upper internodes cambial area is small because here the rings are of small circumference (cf. Fig. 8B).

The space intervening between any two sequences indicates the increment of cambial area in a season and it will be observed that in the main axis this

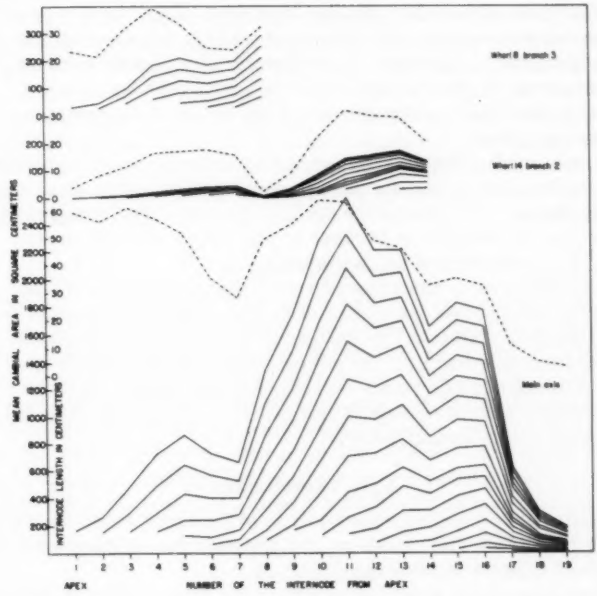

FIG. 11. Type 1 sequences of mean cambial area for two branches and the main axis of tree XA58.9. Broken lines represent internode length, solid lines mean cambial area of individual annual rings.

increases above the sequence in the 9th internode. This coincides with release of the tree, the eight uppermost sequences having been formed after 1950.

Of the two branches portrayed that from whorl 8 developed entirely after release of the tree, and the mean cambial area was determined, as in the corresponding portion of the main axis, largely by the basipetal increase in cambial circumference and to a minor extent only by internode length. The uniform spacing of sequences indicates a steady annual increment of cambial area with no evidence of secondary suppression. This is expected in the 8th whorl of a tree in open conditions.

For contrast we include a branch from whorl 14 which began its development while the tree was in a 6′×6′ plantation. The lowermost sequence, with two internodes, was laid down when this branch was in the second whorl, the next with three internodes, in the third whorl, and so on. The rings laid down in the 5th, 6th, and 7th whorls show small annual increments, which we may put down to suppression of cambial activity in these positions in a close plantation tree. After removal of the neighboring trees there is some temporary improvement in annual increment but by the time the branch was overlaid by 9 or 10 whorls secondary suppression set in and increment of cambial area was negligible in the last 2 or 3 years of the branch's existence. The final result was restricted cambial development throughout the branch. Its distal internodes developed only after it was overshadowed by many whorls; its basal internodes, though initiated when in a more favorable position on the tree soon came to be suppressed by neighboring trees and later by overlying whorls of branches. The ultimate cambial area in basal internodes of whorl 14, with 11–14 annual rings, was distinctly less than in whorl 8, with 5–8 annual rings, although the lengths of the internodes involved were at least as great.

In trees that were always in the open the lower branches achieved greater cambial area because of the normal increments made while they were within 9 or 10 whorls from the top of the tree. In the tree that remained in a close plantation they achieved less because of continued restriction from the time when they were overtopped by five whorls.

Mean cambial area is the basis of reference for both specific increment of wood volume and specific increment of cambial area, the two manifestations of cambial growth activity. The former is a measure of production of wood per unit amount of cambium. It is equivalent to ring width and has been discussed at length in the preceding paper of the series (5). The latter is a measure of production of new cambium per unit of existing cambium, and will be discussed in the following paper.

VII. Conclusion

The present paper completes the analysis of the linear components of growth in four red pine trees and considers the distribution of integrated growth in terms of wood volume increment.

In the previous paper it was shown that the distribution of radial growth in branches is subject to the same influences as that of the main axis, but is modified by the position of a branch on the tree. The distribution of tangential

and longitudinal growth is here interpreted according to the same principles that were applied to radial growth. Increase in circumference, being a product of cambial activity, has a close affinity to radial growth, while longitudinal growth, confined to the apex of any axis, is less affected by branch position in an open grown tree. This may be attributed to the fact that at all stages of its development a large portion of the periphery of a tree in the open is well exposed.

Release of a tree from suppression by its neighbors affects particularly the group of branches lying in about the 5th to the 10th whorl at the time of release together with those internodes of the main axis that lie below these branches, and imparts to the subsequently developed portions of the tree the characteristics of growth in the open.

Interpretation of the distribution of the total annual production of wood is complicated by the fact that it is not directly referable to the current activity of either cambial or apical meristem but depends largely on the past activity of both. If this is kept in mind, however, an insight into the control of integrated growth of trunk and branches may be obtained.

The analysis of branch growth reveals that branches in the upper, well-exposed portion of a tree repeat the patterns of the main axis while configurations of growth in the existing branches are affected by the changing position of the branch relative to the apex of the tree as its successive internodes develop. The value of branch analysis for purposes of investigating biological controls of growth derives from this changing relative position. Individual branch internodes of a single tree provide a great variety of biological entities, genetically identical but each differing from others in its relation to both intrinsic and extrinsic factors. They therefore afford good potentialities for the investigation of factorial control of growth, and the analysis that has been presented provides a basis of reference for such an investigation. Further data are on hand for an inquiry into the nature of some of the factors controlling cambial growth and will be presented in a subsequent paper.

Acknowledgments

The authors acknowledge with gratitude the continued support of the Committee on Extra Mural Research Projects of the Department of Agriculture that has made possible the publication of this paper. This contribution rests on the programme of studies of the growth of red pine that was established by the late Professor G. H. Duff and we owe a debt to all who contributed to that project. The specimen trees were available because of the courtesy extended by the Ontario Department of Lands and Forests, the Department of National Defence, and the Petawawa Forest Experiment Station. The late Professor B. W. Kelley of the University of Waterloo superintended the field work involved.

References

1. DUFF, G. H. and NOLAN, N. J. 1953. Growth and morphogenesis in the Canadian forest species. I. The controls of cambial and apical activity in *Pinus resinosa* Ait. Can. J. Botany. **31**, 471–513.

2. DUFF, G. H. and NOLAN, N. J. 1957. Growth and morphogenesis in the Canadian forest species. II. Specific increments and their relation to the quantity and activity of growth in *Pinus resinosa* Ait. Can. J. Botany, **35**, 527–572.
3. ENGLE, L. G. and SMITH, N. F. 1950. Red pine growth ten years after thinning. Papers Mich. Acad. Sci. **36**, 101–109.
4. EVERSOLE, K. R. 1955. Spacing tests in a Douglas fir plantation. Forest Sci. **1**, 14–18.
5. FORWARD, D. F. and NOLAN, N. J. 1961. Growth and morphogenesis in the Canadian forest species. IV. Radial growth in branches and main axis of *Pinus resinosa* Ait. under conditions of open growth, suppression and release. Can. J. Botany. This issue.
6. FRIESNER, R. C. 1944. Correlation of elongation in primary, secondary and tertiary axes of *Pinus strobus* and *P. resinosa*. Butler Univ. Botan. Studies, **6**, 1–9.
7. GUILLEBAUD, W. H. 1951. Norway spruce spacing plots at Wessling, Bavaria. Forestry, **24**, 121–126.
8. HUMMEL, F. C. 1949. Interim note on thinning study in young pine in East Anglia. Forestry, **23**, 78–79.

NOTES

NOTE ON THE EFFECTS OF CALCIUM CONCENTRATION IN CULTURE SOLUTIONS UPON THE LEAF AND WHOLE PLANT ABSORPTION OF IRON AND MANGANESE BY DWARF KIDNEY BEAN

C. D. TAPER AND W. LEACH

The following reports a study of the absorption of iron and manganese by dwarf kidney bean, *Phaseolus vulgaris* var. *humilis*, grown for 30 days in water culture according to procedures used in a previous study (1). The culture solution provided a wide range in iron and manganese content, and two concentrations of calcium. The one new feature in the present paper is that spectrophotometric analyses were made on whole plants as well as on leaves, whereas in the earlier study the analyses were on leaves only.

Data are given in Table I (p. 438). A depressant effect of iron or manganese upon the absorption of the other, and of calcium upon both, is evidenced. An increase in the concentration of either iron or manganese relative to the other in the culture solution resulted in a depression of the accumulation of the other in both leaves and whole plant. A similar effect in respect of both metals, regardless of their concentrations and the ratio between them in the culture solution, accompanied an increase in the calcium concentration in the solution. Further, iron: manganese ratios in the culture solution were related to the presence or absence of deficiency symptoms apparent as leaf chloroses. It will be noted that the average dry weight of plants exhibiting deficiency symptoms tended to be low. Calcium level seemed a factor determining the optimum iron: manganese range supporting the growth of healthy plants. It will be noted that this was narrowed to 2.0 in the case of high calcium, 143 p.p.m.

Although the results with whole plants represent new information, the conclusions are essentially those reached by analyses of leaves only; and the foregoing support the conclusions in the previous paper (1).

The data show that both iron and manganese are required for healthy plant growth and, for a given set of conditions, indicate the necessity for a minimum amount of any one metal, iron or manganese. For instance, the minimum content of iron for healthy growth in solutions containing high calcium, 143 p.p.m., appears to be somewhere between 0.458 and 0.740 mg/g dry wt., whereas with low calcium, 42 p.p.m., it is somewhere between 0.299 and 0.476 mg/g dry wt. In general, the new data suggest that the effects of the ions in the culture solutions are effects on uptake as such and not simply upon accumulation in the leaves.

1. TAPER, C. D. and LEACH, W. 1957. Studies in plant mineral nutrition. III. The effects of calcium concentration in culture solutions upon the absorption of iron and manganese by dwarf kidney bean. Can. J. Botany, **35**, 773–777.

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TABLE I

Effects of calcium concentration in the culture solution upon the absorption of iron and manganese by dwarf beans

Culture No.	p.p.m. in culture solution				Symptoms*	Content, mg per g dry material						Average plant dry wt., g
						Leaf			Whole plant			
	Ca	Fe	Mn	Fe:Mn		Fe	Mn	Fe:Mn	Fe	Mn	Fe:Mn	
1	42	5.00	25.00	0.2	−Fe	0.165	0.664	0.25	0.299	1.576	0.19	1.49
2	42	10.00	50.00	0.2	N	0.240	0.765	0.31	0.490	2.781	0.17	1.90
3	42	0.05	0.10	0.5	−Fe	0.090	0.215	0.42	0.090	0.215	0.42	1.20
4	42	5.00	10.00	0.5	N	0.242	0.417	0.58	0.476	0.957	0.49	2.17
5	42	5.00	5.00	1.0	N	0.265	0.287	0.92	0.548	0.578	0.95	2.10
6	42	10.00	5.00	2.0	N	0.410	0.239	1.71	0.825	0.422	1.96	2.55
7	42	25.00	5.00	5.0	N	0.599	0.162	3.70	1.227	0.246	4.99	2.25
8	143	5.00	25.00	0.2	−Fe	0.126	0.501	0.25	0.234	1.287	0.18	1.38
9	143	10.00	50.00	0.2	−Fe	0.211	0.598	0.35	0.249	1.259	0.19	1.29
10	143	0.05	0.10	0.5	−Fe, −Mn	0.070	0.160	0.44	0.070	0.160	0.44	0.67
11	143	5.00	10.00	0.5	−Fe	0.172	0.386	0.40	0.367	0.821	0.45	1.68
12	143	5.00	5.00	1.0	−Fe	0.208	0.234	0.90	0.458	0.472	0.97	1.75
13	143	10.00	5.00	2.0	N	0.313	0.174	1.80	0.740	0.385	1.93	3.49
14	143	25.00	5.00	5.0	−Mn	0.439	0.101	4.34	0.980	0.204	4.80	1.88

* N, normal; −Fe, iron deficiency; −Mn, manganese deficiency. Combinations of these indicate varying results within a treatment.

G. H. DUFF MEMORIAL SYMPOSIUM ON DEVELOPMENTAL PHYSIOLOGY[1]

INTRODUCTORY REMARKS BY THE CHAIRMAN

G. KROTKOV

If one examines the opportunities for graduate work in Plant Physiology in Canada, one will find that a prospective student can go to a number of Canadian Universities for his complete training up to Ph.D. This, however, is something new. Until the last 10–15 years there were only two universities in Canada which provided such facilities. These were the University of Toronto and McGill.

As professor of Plant Physiology at the University of Toronto since the early twenties, the late Dr. G. H. Duff has been training Canadian plant physiologists for the last 40 years. His students now occupy positions in various academic and Governmental institutions from coast to coast and through them he has exerted a strong influence on the development of this field of botany in Canada.

His influence, however, has been not only through his students. It was Dr. Duff who, about 10 years ago, invited a number of Canadian plant physiologists to come to Toronto for a day of informal discussions. The meetings were so successful that it was decided to hold them every year. They were held informally in subsequent years at various Governmental and academic institutions until, at our last year's meeting in Saskatoon, a Canadian Society of Plant Physiology was organized.

While the first meeting of Canadian Plant Physiologists was held at the University of Toronto, for each successive gathering we went to some other institution. Now we have returned to Toronto for the second time. In view of the contributions of Dr. Duff to the field of Plant Physiology in Canada, your Executive thought that it would be appropriate at this meeting to have a symposium in his honor. The question was raised: what ought to be the subject of this symposium?

Very early in his career Dr. Duff became interested in developmental physiology. Over a period of years he and his students studied respiratory ontogeny of wheat leaves and its relation to leaf metabolism in general. In later years he became interested in the enzymic changes taking place during ontogeny, as well as in the problem of growth and morphogenesis in Canadian forest species. In view of these interests of Dr. Duff, it was decided to hold a symposium in the field of developmental physiology. We have with us three speakers, who will discuss three different aspects of this subject:

Dr. F. C. Steward from Cornell University will speak on the "Organization and Integrations: Some Outstanding Problems in Cells and Plant Physiology".

[1]A symposium of the Canadian Society of Plant Physiologists held on June 2, 1960, at the Department of Botany, University of Toronto.

Dr. E. T. Bünning from the University of Tübingen will speak on the "Endogenous Rhythms and Morphogenesis".

Dr. G. A. Setterfield from the National Research Council in Ottawa will speak on the "Structure and Composition of Cell Organelles in Relation to Growth and Development".

VISTAS IN PLANT PHYSIOLOGY: PROBLEMS OF ORGANIZATION, GROWTH, AND MORPHOGENESIS[1]

F. C. STEWARD

Preamble

At the First Annual Scientific Meeting of the Canadian Society of Plant Physiologists your present speaker presented, extemporaneously, a talk in which certain problems with which he had been, and is still, concerned, were passed in review. The purpose of this talk, as delivered, with its illustrative material was to present the challenge of plant physiology for those who will interpret the behavior of cells and organisms as highly organized, but closely integrated, living systems.

A feature of the current scene is a whole new and thriving subject which has come to be called "molecular biology", beside which even the still fresh but somewhat older one of "comparative biochemistry" is already somewhat staid. Thus the modern science of genetics, which touches all branches of biology and was born with the rediscovery of Mendel's Laws in 1900, stimulated a comprehensive approach to biological problems which easily crosses subject matter boundaries. It is in this trend that the sciences of comparative biochemistry and biochemical genetics developed, for these recognize that the basic enzyme catalyzed reactions of life are often similar from mouse to man, and that they in turn may be controlled and regulated by the enzymes which are gene-determined. It is a dramatic achievement that some account may now be given, through the "molecular biology" approach, of the way in which the blueprint of an organism is laid down, as it were, in "information centers" composed of DNA molecules which control subsequent metabolic events.

None of this is either denied or minimized. Nevertheless, this trend always stresses the similarities in nature, it deals with the properties that are basic and common to all living organisms. This becomes more apparent the more organisms are treated in terms of their individual chemical reactions and the properties of subdivided bits of the vital machinery, i.e. the particles which it is now so fashionable to study and the cell-free preparations in which it is the aim to emulate any given physiological activity which may be under investigation. Were this all, the modern botanist and plant physiologist might be content to lose his identity in one or other of the newer and fashionable disciplines to which reference has been made. But this need not, and should not, be so. For in the techniques of subdivision and dissection the first casualty is that most distinctive physiological property of all—the capacity for growth; for this is a feature of the entire organization. It is in the culminating study

[1]Manuscript received December 27, 1960.
Contribution from the Department of Botany, Cornell University, Ithaca, New York. Paper presented at the G. H. Duff Memorial Symposium on Developmental Physiology, University of Toronto, Toronto, Ontario, June 2, 1960.

of growth—the summation of all the functions of cells and tissues—that cell and plant physiology really comes into its own.

Even after the living system has been subdivided into its most minute parts, its cells, particles, genes, DNA molecules, enzymes, proteins, etc., we might still know little about how the organism functions, how it grows and develops. As little, in fact, as one might know about the turmoil which is New York City by digging up a cemetery, and obtaining a glimpse of the skyline, on the one hand, or of the subway on the other. (The parallel is not inapt, since even a single carrot cell contains of the order of 10^{15} water molecules, and for every million or so of these a protein molecule, a veritable molecular skyscraper exists, but even at this complexity a cell of carrot allows for 10^9 individual protein molecules of an average size of about 64,000.) A great challenge of modern biology then is to elucidate the problems of growth in such a molecular organization, for it is in the ability to grow that the gap between the animate and the inanimate is most apparent. The crucial task is here not merely one of dissection and of subdivision—not merely the recognition of properties of smaller and smaller units of the living system or of the effects of single variables or of single processes in isolation from all the rest—but it is primarily a task requiring synthesis, of showing how these separate entities, structures, and functions combine together to form the integrated harmonious whole which we recognize as the living cell, or organism.

In this field, differences are as important as similarities. While comparative biochemistry recognizes the similarity between *Chlorella* and an oak tree, there is obviously some point of essential difference between them! The cells of the phloem and the xylem, cut off from the same cambial element, may each receive the same genetic endowment, but what determines their respective trends of development so that, within this same genetically predetermined range, they perform such different functions in the organism as a whole? And who could contemplate the dramatic events of photoperiodism upon the form, the function and reproduction of plants without recognizing the underlying problem of morphogenesis which is to determine how such a complex organization responds by its growth in so dramatic a fashion to such direct and apparently simple stimuli? The plea, therefore, was and is again made that plant physiologists should exploit, in the modern day with its unparalleled tools and techniques, the distinctive and challenging features of their subject and of its experimental material. In so doing, the interpretation of cellular organization, of growth, development, of differentiation, and of morphogenesis will inevitably loom large, and in pursuing this task through the study of higher plants the subject will have distinctive contributions to make.

While it seemed fitting in a program designed to honour Professor G. H. Duff to stress these points, much of the evidence which clothes the argument with factual support has already been published in one or other of several recent comprehensive accounts of work done in the areas of research from which these thoughts derived (cf. Steward and Mohan Ram (1961) on Determining Factors in Cell Growth: Some Implications for Morphogenesis in

Plants; Steward *et al.* (1961) on Growth Induction in Explanted Cells and Tissues: Metabolic and Morphogenetic Manifestations; Steward (1961) Organization and Integration; Steward and Sutcliffe (1959) on Plants in Relation to Inorganic Salts; Steward *et al.* (1959) on Nutritional and Environmental Effects on the Nitrogen Metabolism in Plants). Therefore, much of the detail may be omitted here, thus permitting one to survey the salient trends which lead to currently challenging tasks of plant cell and developmental physiology.

A major task for the modern plant physiologist is to interpret the role of organization: organization in cells, because all of the cell physiological functions proceed concomitantly and in phase however, we may choose to subdivide and segregate them for purposes of investigation: organization in growing regions, as, for example, of shoot and root, because they owe their properties not only to the attributes of their constituent cells but also to their place in the organization (strikingly different as this is in shoot and root and in vegetative and floral apices), and, last but not least, organization in the fertilized egg or zygote itself, for this contains all the information to produce the entire organism. Here one may now recognize that the behavior of the zygote and even such preceding events as meiosis may not in fact be as uniquely associated with reproductive centers and processes as may have been supposed, for they are beginning to be recapitulated in freed cells stimulated to grow by coconut milk. In short, the extent to which the early events of embryogeny may be subtly determined by the specialized nutrition supplied in the embryo sac and ovule may have been underestimated: not only "nature" but "nurture" are here involved. Thereafter, factors in the environment and throughout development make their impact upon the growing organism, and the interpretation of these morphogenetic events becomes a distinctive and challenging task for the experimental botanist and plant physiologist.

Increasingly, therefore, one sees that the characteristics which may be initially predetermined by the "blueprint" implanted in the DNA of the chromosomes may be modulated or controlled by factors which operate during development. And many of these regulatory effects must have a chemical basis. As plant physiologists exploit these distinctive features of their material they keep their science alive, responsive to modern trends, but also as a distinctive branch of biology. It is both fitting and opportune, therefore, to express these views upon developmental plant physiology dedicated to one who was especially interested in this field. And it is particularly fitting to do this at a time when there is an increasing preoccupation with the purely chemical and molecular basis of biology. The so-called science of "molecular biology" has markedly advanced our knowledge of metabolism; as yet it has taught us virtually nothing about the problems of organization. Such problems begin where "molecular biology" ends. Indeed, if the sole preoccupation of the plant physiologist were to be with molecules, then the subject should properly be a branch of chemistry. But many major problems of biology—problems of growth and development, of differentiation and morphogenesis—are concerned with systems which operate at levels of organization far

greater than the molecular, even though their regulatory control may be mediated by comparatively simple chemical compounds. Such problems of organization now arise in almost every modern phase of plant physiology and some salient examples are cited below.

Trends in Nutrition in Relation to Growth

The first impact of chemical knowledge upon plant physiology came through the need to understand nutrition—inorganic plant nutrition—as the basis of agricultural science. The essential landmarks are well known. From Aristotle to Van Helmont, the relative roles of earth, air, and water in the forming of plant substance were first accepted, then disputed. These great questions were to be resolved successively in 1699, by Woodward, who stressed the role of dissolved substances in what were essentially the first water culture experiments on growth; by the discovery of photosynthesis and the fixation of carbon in the late 18th century and the recognition by de Saussure at the outset of the 19th century that water also enters as a reactant into the processes of organic nutrition through photosynthesis.

The recognition of essential inorganic nutrients of plants emerged as the distinctive contribution of the 19th century with the stress on what later came to be recognized as the major nutrient elements in the list of 10, widely accepted at the turn of the 20th century. This list of 10 elements were to become the basis of the familiar three salt culture solutions (with Fe added) and of the device, now seen to be ingenuous, of defining the best nutrient solution for growth in the respective concentrations of three main nutrient salts. Despite prior indications that other elements might be needed for fuller and more complete plant nutrition, the present knowledge of trace elements in plant nutrition is an outstanding contribution mainly of the second quarter of the 20th century, and this culminates in the enzymological approach, which has permitted the role of some of the elements to be explained in these ways.

But the history of plant nutrition well illustrates the main thesis of this discussion. Dramatic as are the events referred to above, the trend is again essentially descriptive, in chemical terms, of the essential requirements for the growth of plants, and so long as the description necessarily concentrated on the recognition of these requirements, one by one, the science of plant nutrition lacked its essentially modern challenge. A true philosophy of plant nutrition emerges not only from an understanding of what each element may uniquely and separately do (and knowledge does not permit even the requirement for potassium to be rigidly explained in this way!) but also how they *collectively* function, not in isolated reaction systems, but in the growing cells and meristems of plants. Here *interactions* between elements are as important as their single effects, as, for example, between K, N, and P; Fe and Mn; Ca and B; Cu and Mo; but even this is not all.

Strangely the history of plant nutrition was slow to recognize that nutrition was not a rigid requirement but is itself a function of environment. Interactions of light and potassium and nitrogen in nutrition, between zinc and insolation, boron and length of day, foretold the shape of things to come.

Mint (that recently neglected but yet classical plant of plant physiology) has yielded one of the first documented cases which show that the inorganic requirements for "best" growth vary drastically according as the growth is that of a long-day or a short-day plant (Crane, *cited from* Steward *et al.* 1959). *Mentha piperita* behaves as a calciole species under long days, requiring a high ratio of Ca to K to use to the full its nitrate supply; but under short days it is a calcifuge species, requiring a higher ratio of K to Ca (work of Crane as summarized by Steward *et al.* 1959). And now, as also may be seen from the study of mint, such variables as photoperiodism and thermal periodism carry their effects beyond the gross morphology of the plants in question (see Fig. 1) to many phases of their intimate metabolism (Steward *et al.* 1959). In particular, the metabolism affected is that of the keto and amino acids.

All this clearly means that the future study of plant nutrition must go far beyond the separate investigation of individual nutrient effects. The function of nutrients, which is to nourish the growing organism and provide for the needs of the centers of growth (i.e. primarily the apices of shoots, vegetative and floral, and roots) must vary vastly in accordance with the environmental effects that determine how they grow. The concept of a "best solution" for this or that plant is thus outmoded, for increasingly it is necessary to recognize that any, or all, of the nutrient requirements may be graduated in accordance with fluctuations in other variables. This obviously poses great questions in the design of experiments. Even though modern engineering may control the environmental variables which affect growth, how can one obtain all the relevant data (nutritional and metabolic) on all the necessary parameters in a given case, and, having done so, how can these complex interacting effects be sorted out? Looking ahead, it seems more important, in this area of the physiology of growth and nutrition, to work out methods for the proper conduct of this kind of investigation and to estimate the ancillary resources needed, than to assume that the problem is solely one of controlling the external environment of plants. Unless this is done, resources may well be as wastefully and as unprofitably used at the present as they were earlier, when it was thought enough to determine the composition of a "best" nutrient solution for the growth of a specific plant in a medium whose osmotic pressure was established at a predetermined level, largely at the caprice of the investigator!

In short, in the science of plant nutrition one should now recognize that nutrients nourish the plant which grows, and, as the facts of morphogensis show, the superimposed effects of environment create a different kind of plant as they interact with the organized growing centers. No longer should we assume that the same fixed nutrients, in the same proportions, are needed for all the different tissues and organs of the plant body, for the need for nutrients may also vary with the way the cells or the organs grow. (For example, phloem tissue explants from the storage root of the carrot will grow apace in a solution so free of calcium that the organized root tip could not flourish, i.e. a basal medium made calcium-free with the addition of calcium-free coconut milk, i.e. oxalated coconut milk with excess of oxalate ions present.) Therefore, we should no longer assume that the requirements for

the individual nutrient elements is a basic and overriding need of a species or a variety, for this is now appearing to be also a genetic characteristic. In the work of Munger (Pope and Munger 1953 *a* and *b*) two examples are now known in which single genes determine the level of requirement for specific nutrient elements (Mg in one case; B in the other). In fact, this speaker has always thought that it is not accidental that new examples of trace element nutrient abnormalities in the field are apt to occur where there is the use of *highly selected genetic strains of cultivated plants grown in habitats and under environmental conditions which were not those used during their initial selection.* So often it is the introduced cultivated plant which proves to be subject to nutritional imbalance which the climax natural vegetation in the same environment withstands.

Thus the future of nutritional plant physiology does not lie alone with the biochemical and descriptive approach, valuable as that is, for a comprehensive philosophy of plant nutrition can only emerge as one attempts to see how the whole complex machinery of the plant body and its organization is nourished in a fluctuating environment that modifies both its growth and its requirements.

Trends in Cell Physiology

The foundations of plant physiology as a separate discipline were largely laid in what may now be recognized as the beginnings of cell physiology. The enunciation of the cell doctrine (by Schleiden and Schwann 1838–39), the recognition of protoplasm (Von Mohl 1846), the discovery of plasmolysis leading to its recognition as a special case of osmosis (Naegeli 1855; Hofmeister 1867; de Vries 1877), the investigation of membrane phenomena with both artificial and natural membranes (Traube 1867; Overton 1890) helped to lay the foundations of plant cell physiology, contributed to the physicochemical theory of solution, and also led to the now vast literature on osmotic relations of cells and upon cellular permeability.

In retrospect, one may recognize that plant physiologists were first, and of necessity, preoccupied with the attempt to interpret cells as they are—rather than the processes by which they grew and came to be—thus the classical equilibrium theories of osmotic regulation and passive permeability emerged. Although this trend had enriched plant physiology and provided a nucleus around which so much of plant physiology as a distinct discipline came to be built, one can now see its essential short-coming, for too often cells came to be regarded as passive systems, responding in a simple way to the dictates of true equilibrium. Especially should growing cells be seen as systems which are not in true equilibrium with their environment but in an intensely dynamic manner, and through the creation and maintenance of their own morphology, they use processes of metabolism and nutrition to increase free energy and to maintain relations with the environment which could not obtain at equilibrium alone. It has been said that organisms "feed on negative entropy". Thus the understanding of what they do becomes a problem of their organization and not only of selected processes considered in isolation.

This was appreciated for the problem of salt accumulation with which the present speaker, along with many others, was first concerned. The detail,

FIG. 1 (above). (A) Mint plant (*Mentha piperita* L.) grown on short days (8 hours) for 43 days. (B) Plant grown on long days (18 hours) for 24 days in full nutrient solution in the greenhouse.

FIG. 2. Electron photomicrograph of cellulose in the wall of *Valonia* (from Steward and Mühlethaler 1953). (A) Randomly oriented fibrils in the newly formed cellulose at a naked surface of an aplanospore. (B) Cellulose in the later formed wall of a mature vesicle, showing fibrils in parallel arrangement in each of three lamellae: each successive lamella is laid down in a different direction.

recently summarized (Steward and Sutcliffe 1959) need not be recapitulated here. Suffice it to say that, today, hardly anyone would seriously attempt to explain the remarkable facts of dynamic secretion or active and selective accumulation of ions in cells wholly apart from the intervention of the processes of growth and metabolism which furnish the necessary energetics. Opinions may, and do, differ on how the biological machinery works, on just how it utilizes its resources of chemistry and metabolism to bring about the events which do occur, but few would claim that all this can be determined solely by the fixed and passive properties of membranes. The *de novo* accumulation of ions in the aqueous phases of cells still seems to be essentially a function of the development of the cell, an essential characteristic of it at a period of its own growth and development and, in that sense, de Vries' idea of the tonoplast as an organ of internal secretion is not inapplicable. However, the metabolic property which best indicates the ability of the cell to accumulate ions *de novo* is not alone its ability to maintain a high rate of aerobic respiration, as shown by carbon dioxide output, but its protein synthesis as the evidence that the energy so liberated may be canalized to the useful and constructive processes of growth. The evidence behind these broad conclusions has been summarized and reviewed (Steward and Sutcliffe 1959).

However, the ultimate question is still as obscure as ever. Since the early speculations upon the cell as a metabolic machine that directs the energy of metabolism and respiration into salt accumulation, as but one of the many processes in growing cells which use and require energy, much has been learned about how cells negotiate such energy transfers. Phosphate bond energy is now universally recognized as the main currency through which such transactions are negotiated, but one still needs to know precisely the way in which energy released from respiration and locked up in these compounds is donated at sites where the activated movements that result in salt accumulation occur. Although the case for activated water movements in plants may not be so generally accepted, it seems nevertheless true that the *de novo* uptake of water is so intimately a part of the pattern of cell growth and development that one could hardly explain the one without the other. In fact, water uptake, ion accumulation, protein synthesis, and high rates of aerobic respiration so frequently vary *pari passu* and so frequently respond similarly to external variables that they all seem to be interconnected at some focal point in the cell. The place where this occurs seems to be the point where the energy of metabolism is linked or canalized to the processes of growth (of which protein synthesis, water intake, ion accumulation, etc. are separate manifestations), and it is of particular interest, as shown below, that chemical growth factors, which make an impact upon the cells' ability to grow in a given nutritional environment, commonly affect all these physiological processes concomitantly.

However, the point which is relevant to this discussion is as follows. Knowledge in this area is limited by, and future progress requires, an understanding of the cell as a working machine, and this can only emerge from a more intimate knowledge of its complex organization. One might as well try to understand the workings of the internal combustion engine by letting a car roll downhill with the brake off as to understand the active processes of water and salt intake without recourse to how cells grow and metabolize! But all

too often our ideas of physiology and metabolism have been drawn from such cellular systems which either could not, or would not, grow under the conditions in which they were studied.

Dramatic developments may, however, be at hand in the advances now being made in the field of submicroscopic morphology. Electron microscopic studies of cells (the subject of another paper in the symposium) now being carried out by such workers as Porter, Whalley, Mühlethaler, Manton, Setterfield, and others, using methods that permit a study of the cytoplasm, now revolutionize our ideas of the cytoplasm of cells. At the microscopical level it is easy to be impressed with the evidence of organization in cells which is to be seen in the bright field and phase microscope, with the evident signs of intense dynamic activity, shown by Brownian movement and obvious streaming of granules along cytoplasmic strands, which occurs in cells while they accumulate ions. This is evidence enough of their ability to turn metabolic energy into useful mechanical work and is therefore indicative that they may also direct it into osmotic work. But new vistas now appear in the submicroscopic structure of the cytoplasm, and they affect the basis of our concepts of cellular membranes and cellular inclusions. The presence of a distinct endoplasmotic reticulum, densely concentrated at some surfaces and ramifying throughout the cytoplasm, may provide a channel of transport to and fro in the cytoplasm; the possibility of localized and discrete pores in membranes such as the nuclear membrane; the obvious need to understand the role of such diverse, vesicular organelles as the Golgi apparatus, now demonstrable in plants, vacuoles, and plastids; all this endows the work on submicroscopic morphology of the cytoplasm with the greatest interest. It seems that, through a better understanding of the cell's organization and through the harnessing of the descriptive techniques of the electron microscope to experimental investigations, the present stalemate in the interpretation of active transfer phenomena may well be broken.

Again, however, the future of plant cell physiology as a distinctive discipline seems to lie with the closer understanding of cellular organization, and with the attempt to say how, as cells grow and develop, our knowledge of the cell's organization may be linked to modern knowledge of its biochemistry and metabolism.

Trends in Synthesis and Metabolism

There are many examples in which the course of metabolism is not immediately predictable from the enzymes and substrates present in cells. In some cases, e.g. urease, an active enzyme is present even though its role seems not to be involved in the plant or organ in question (cotyledons of Jackbean), for this protein is present as a storage protein in the mature cotyledons, but there is no evidence that they normally metabolize urea. The old idea of protoplasmic control of enzyme action may be illustrated in cherry laurel (*Prunus laurocerassus*) leaves, which contain both amygdalin and emulsin. However, the hydrolytic products (HCN, $C_6H_5.CHO$, and sugar) only arise when by killing, without heat, or rendering the membranes permeable, the substrate and enzyme, which are separated in the normal cell, are allowed to mingle. In another example γ-aminobutyric acid occurs in carrot tissue,

especially the actively growing tissue. It is natural to assume that it could arise from glutamic acid and especially so because the glutamic acid decarboxylase occurs and the equilibrium favors decarboxylation. However, when labeled substrates (C^{14}-glutamine or C^{14}-γ-aminobutyric acid) are furnished to carrot cells, it becomes very clear that the tissue converts γ-aminobutyric acid to glutamine and thence to protein, and C^{14}-glutamine passes to γ-aminobutyric acid virtually not at all (Steward, Bidwell, and Yemm 1958). Therefore, a knowledge of enzymology without knowledge of the way enzymes operate, or not, within the cellular organization can be most misleading.

A problem that clearly involves the role of the organized cell is the synthesis of cellulose and its organization to form a wall. Chemically, cellulose as 1,4-β-glucopyranose chains is well known, and its essentially crystalline structure and crystal lattice have long been known. But the formation of the first formed wall as a weft of tangled cellulose fibrils arranged without order, as at the naked surface of a *Valonia* aplanospore, which is followed by a more orderly and lamellated arrangement of cellulose in distinct layers, in which the directions of the fibrils shift in successive layers so that they cross at well defined angles (see Fig. 2 from Steward and Muhlethaler 1953, and references there cited), presents a still further problem. It is one thing to explain enzymatically how the individual 1,4-β-glucopyranose units are linked by chemical bonds, it is quite another to explain how the protoplasm produces the orderly arrangements of fibrils which constitute a wall. Interestingly enough, fluctuations in the external environmental conditions during growth determine, as in the cotton hair, the structure of the wall (Anderson and Kerr 1958; cf. account of this work in Roelefsen 1959).

The above example (cellulose synthesis and wall formation) is a familiar case of "asymmetric synthesis", i.e. the characteristic property by which living things make use of only a few of the statistically possible structures in the compounds which they make and, faced with alternatives, usually utilize only one. This results in the familiar, but nonetheless remarkable, array of compounds whose physiological role depends not only on their chemical properties, as represented by their functional groups, but by their "molecular architecture" or how they are arranged in space; in short, by the "morphology" of these molecules. This principle operates all the way from compounds which have two enantimorphs, such as alanine, to the sugars and polysaccharides in which synthesis is directed toward a few highly specific structures, out of many, because they are compatible with the role of these substances in cells and organisms.

It is customary to recognize that all such biosyntheses take place in an already asymmetrical protoplasmic environment, for the very enzymatic catalysts which bring these syntheses about are highly asymmetric protein molecules. Nevertheless, this is a feature of the organization of cells and organisms which determines what they can, or can not, do. When pushed to finality, the argument requires that at the origin of life a given self-reproducing molecule possessed the property of "right-handedness" or "left-handedness", and so all subsequent developments must conform to the trend so started by a first set of fortuitous choices. Whether this was, or was not, one can still recognize in the present array of asymmetric biosyntheses the interplay

between chemical possibilities on the one hand and some feature of the organization within which the synthesis occurs on the other.

While modern knowledge of photosynthesis has gone very far indeed to explain the path of carbon en route to sugar, it still does not enable one to draw a blueprint of the factory that will make D-glucose or cane sugar! At some point along the line one has still to usurp for this, some feature which is still an essential attribute of a cell and its organization—even if this is "only" (!) an enzyme. And how much more is this true of protein synthesis?

Plant physiologists and biochemists may indeed take pride in the extent to which such physiological functions as photosynthesis and respiration can now be broken down into their individual reactions. Nevertheless, it is also true that we know but little about the way physiological functions interact and how such diverse processes as photosynthesis, respiration, protein synthesis and breakdown, ion intake, water absorption, utilization of the several inorganic elements, etc., etc. take place harmoniously and in balance in different parts of the same cell's internal geography. To gain the first necessary chemical knowledge of metabolism each process or reaction may first be considered in isolation, but to understand what actually happens in cells we must know, not only how the unit operations work, but how they contribute to the design and smooth operation of the entire metabolic factory.

These objectives cannot be achieved without knowledge of the cell as an organized system, albeit also as a system which grows. One has only to look at modern electron microscope pictures of plastids and mitochondria (Sager 1958) and of the erstwhile optically transparent cytoplasm (Whalley *et al.* 1959, 1960) to see how great these possibilities and problems are. But also at the level of the individual metabolites it is now apparent that the same substance may be doing very different things in different phases of the same cell (cf. Steward, Bidwell, and Yemm on respiration and nitrogen metabolism in carrot cells, 1956), and adequate kinetic methods need to be perfected to reveal these complications and to follow individual metabolites along these different pathways in different inclusions and phases of the cell.

Trends in the Physiology of Cell Growth and Cell Division

The present is one of great interest in the investigation of the underlying causes of cell growth and cell division.

Again the pace of understanding and research started slowly and seems, in retrospect, to have been at times unaccountably slow. After the enunciation of the cell doctrines and while the foundations of cell physiology were being laid, a descriptive period of cellular cytology and morphology ensued, a period which, incidentally, brought forth such great aphorisms as "protoplasm, the physical basis of life"; the nucleus as the "physical basis of heredity", and the realized importance of "self-duplication" in such phrases as "omnis cellule e cellula" and "omnis nucleus e nucleo." While the foundations of modern cytology and genetics were thus being laid in the 19th century, one can also see, in retrospect, the beginnings of what was to become the modern approach to the basic causes of, and the regulatory control of, cell growth and cell division as it is now being exploited in higher plants.

With Darwin (1880) studying tropisms, using the coleoptile of *Phalaris*, the

later hormone concept may be held to have been born, and Darwin also may be held to have ushered in the great wave of activity concerned with what are now known as auxins. This trend reached a landmark with the discovery in 1934–35 of indoleacetic acid, an achievement which resulted from the pioneer works of Went, Thimann, and Kogl. Perhaps, thereafter, progress became unduly tied to ideas which were linearly descended from the discovery of indoleacetic acid, and it was not until some 20 years later (with the discovery of the nitrile of IAA, by Jones *et al.* (1952), with the first chromatographic recognition of other auxines by Bennet-Clark (1953) and later by Luckwill (1959), Nitsch (1955), and others, and with the renewed interest aroused by the gibberellins, discovered years before in Japan, as described historically by Stowe and Yamaki (1957)) that ideas upon the non-nutrient, catalytic agents that regulate the growth of plant cells became more flexible.

Meanwhile the agents and catalytic machinery which primarily induce, or regulate, growth by cell division were strangely slow to appear. As may be seen in Gautheret's major work on plant tissue culture (1959), Haberlandt foresaw as early as 1902 the use of cell and tissue culture techniques, and he worked and speculated on the cell division stimulating substances, or hormones (Haberlandt 1921) which were to become even more credible at a much later date. Despite the advances made by such pioneers as Robbins, White, and Gautheret, who exploited the newly inaugurated aseptic tissue culture procedure, using explanted organs (roots) or tissue explants, real progress toward the specific agents that induce cell division had to wait until many problems of nutrients and vitamins had been solved. But now one can see the gains to come from a full understanding of the chemical machinery which either suppresses, or unleashes, the innate ability of cells to grow.

In our work in this field all the advances that have been made through work on tissue cultures were first motivated by the desire to have a suitable system with which to study the salient problems of cell physiology mentioned above. This system needed to be composed of uniform cells, be readily available in its quiescent state, and capable of being used alternatively in a condition of non-growing, resting metabolism and, at will, in a state of rapid growth by cell division. It was the hope that, in this way, one could understand the distinctive differences between cells that can grow and those in which the metabolism runs to waste, and also to understand more about the means by which the growing cell is able to link its metabolism to such energy-requiring events as protein synthesis and the active processes of water and salt intake. It was in this context, recognizing the role of the organized growing cell, that work on the aseptic culture of carrot tissue stimulated to grow by cell division came about and the reversible stimulus to control such growth, through the use of coconut milk in the medium, was found.

From such work (and that of other laboratories) one can now formulate the following general ideas. Although, as a whole, green plants are fully autotrophic organisms, this may not be true of the individual cells, or organs. Even the embryo of angiosperms, formed in the special environment of the embryo sac, and the ovule is subject in its first and critically formative period to a highly specialized form of nutrition, elaborated by the parent sporophyte, and while it is subject to this heterotrophic nutrition it passes through an

intensive period of development. The environment of the egg is the embryo sac, and the latter grows at the expense of the nucellus but, in angiosperms, the stimulus to grow which is conveyed to the often triploid fusion nucleus from which endosperm forms, even outstrips that conveyed to the zygote itself. By the growth of endosperm (in its free-nuclear liquid or cellular form) there is laid down, in advance of requirement, a potent nutrient source for the developing embryo. One now sees that this nutrient source not only contains the special organic nutrients which the embryo receives but, early in its development, the powerful, non-nutrient stimuli which maintain active cell division. (For summary of this and the following material, see Steward and Shantz 1959; and Steward and Mohan Ram 1961.)

The significance of much of the use of such fluids as coconut milk (the liquid endosperm of the coconut) and the fluid from the vesicular embryo sac of the immature horse chestnut fruit or extracts of corn (*Zea*) in the milk stage to induce cell division is that all of these sources normally nourish immature embryos. The dramatic result is that these fluids or extracts can intervene in the metabolism of otherwise resting, non-growing cells and induce them to grow rapidly again. It is, as it were, as if a metabolic clutch has again put the engine of growth in gear! But not all resting cells respond to coconut milk alone, some require a synergistic combination of coconut milk and one of a now large number of known synthetic growth regulators, of which 2,4-D was the first known example.

Therefore, over and above known nutrients and vitamins, the capacity of cells to respond by growth may be limited by access to exogenous stimuli. These stimuli to cell division reside peculiarly in endosperms of immature fruits and seeds, and they in turn work in conjunction with synergists, which accentuate their effect, and antagonists which suppress it. Examples of all these effects, with citations to the relevant literature, can be found in the following papers (Steward and Shantz 1959; Steward and Mohan Ram 1961).

The differences between resting, quiescent cells and their actively growing counterparts should now be noted. This is conveniently shown by the contrast between the cells of carrot, potato, and banana fruit as they occur in the storage organ in which they are formed and as they can now be grown as free cells in a liquid medium (Steward, Mapes, and Smith 1958). Even the most casual study of Fig. 3 stresses that these are organizationally very different cells. However, when seen in life, the comparative absence of motion in the storage cell and the intense Brownian movement and protoplasmic streaming of the cultured cells emphasizes the contrasts between the two systems. Too much of current plant physiology relates to the cells in the former state (with the metabolic fires banked down and the engines of growth out of gear) and too little relates to cells in the latter state with the organization working at its optimum, making maximum demands for nutrients on the environment and directing the metabolic machinery in the direction of growth. What one now needs to know is how all the processes of metabolism and how all the separate physiological functions combine in this complex morphological system to produce the harmonious working of the whole.

It is for these reasons that plant physiologists should pay increasing atten-

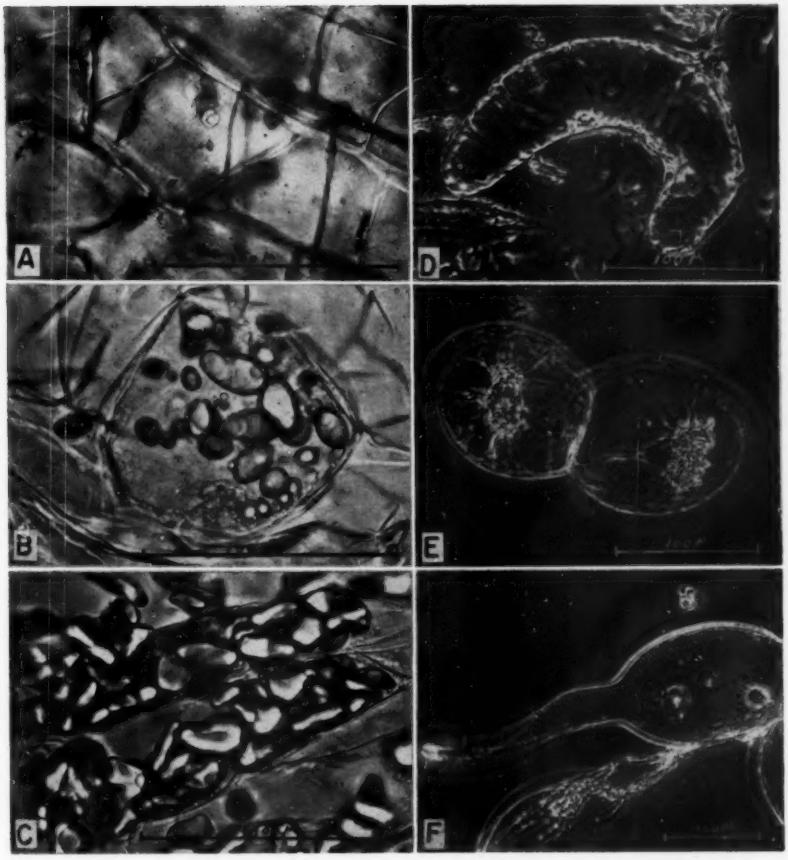

FIG. 3. Contrasts between quiescent cells and their active counterparts growing free.
(A) Resting cell of the secondary phloem of carrot root, showing carotene in chromatophores.
(B) Resting cell of potato tuber showing abundant starch grains.
(C) Resting cells of banana fruit pulp showing abundant starch grains.
(D) cf. A; free cell of carrot, grown in liquid medium containing coconut milk (10%).
(E) cf. B; free cells of potato tuber, having grown in liquid medium containing coconut milk (10%) + 2,4-D (2,4-dichlorophenoxyacetic acid) at 6 p.p.m.
(F) cf. C; free cells of banana fruit activated to grow by coconut milk and napthalene acetic acid.

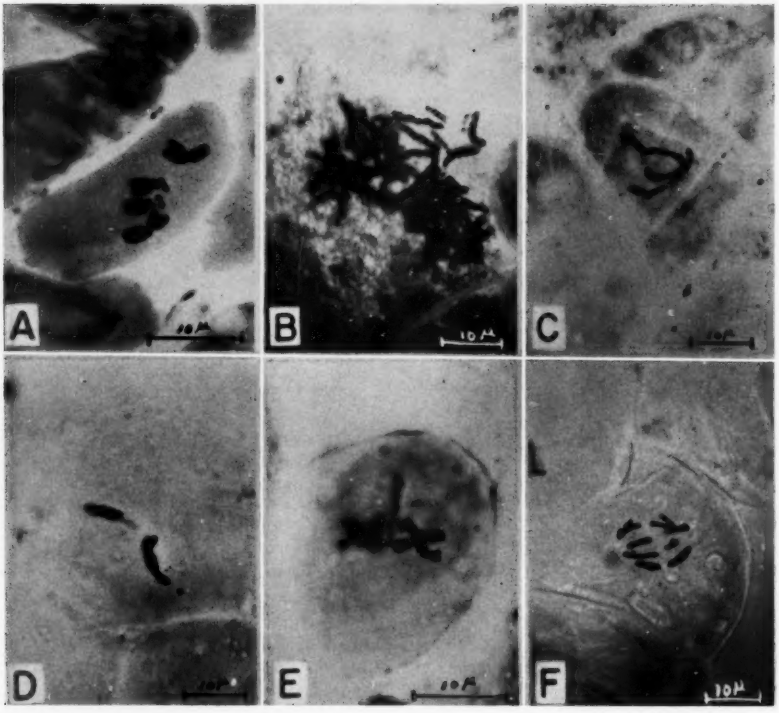

FIG. 4. Chromosomes of *Haplopappus gracilis*.
(A) Chromosomes in metaphase in root-tip cell ($2n = 4$). Similar figures are of usual occurrence in cultured cells.
(B) High degree of polyploidy in cultured cell.
(C) Haploidy in cultured cell.
(D) Somatic pairing in cultured cell.
(E) Pseudochiasma in a cultured cell.
(F) Chromosome breaks in cultured cell. C, D, and F: grown in a basal medium supplemented with coconut milk and casein hydrolyzate. B and E: same as above plus NAA.

tion to the problems of cellular organization, for even in a single cell the same metabolite may perform quite distinct metabolic functions. In fact, a recent analysis of protein metabolism and respiration in the cells of growing carrot cultures only became intelligible as one recognized that anabolic and katabolic metabolism occurred side by side and that the coconut milk stimulus to cell division operated in two ways. First, the coconut milk accentuated over-all protein synthesis and, second, it accentuated the rate of turnover and the consequent usage of the breakdown products as carbon substrates in respiration (Steward, Bidwell, and Yemm 1956). Thus the effect of these stimuli to growth and cell division will need to be precisely located and described with respect to the organization of the cell in which they intervene.

But the investigation of the chemical induction of cell growth and cell division has, in this laboratory, recently taken another dramatic turn. An ultimate goal has long been the culture of single, or free, cells of higher plants. As early as 1902, Haberlandt saw the possibilities for the interpretation of differentiation and development if this could be achieved (cf. the account of this work in Gautheret, 1959). A fortunate item of technique yielded a purely mechanical means by which carrot cells could be obtained freely suspended in the liquid medium which contained cultures growing under the stimulus of coconut milk. This device initiated investigation to show how the free cells grow and to demonstrate their morphogenetic potentialities.

The first result was that the isolated, freely suspended cells grow and attain a multicellular, colonial, condition by means that would never be suspected from their behavior in the higher plant body or in a preformed tissue mass. These unusual modes of cell multiplication, which include budding, formation of filaments which grow terminally, internal segmentation of multinucleate giant cells as well as equational division of more of less isodiametric cells, have all been described. What makes one cell adopt one of these means whereas others from the same source and in the same suspension behave differently is not known. The following point is, however, relevant to the present discussion.

When freed from the tissue in which it is formed initially, when prompted to grow in isolation from its neighbors, a phloem parenchyma cell of the carrot root displays an unexpected range of growth forms (Steward *et al.* 1958). It is as though the presence of the cells in the organized tissue mass restrains their growth and behavior, for what they then do is largely a function of the tissue as a whole. However, when they are free, the same cells respond in a far wider range of independent ways and produce the observed range of growth forms and cellular behavior. Thus, what a given free cell does is a function of its innate capacity for growth, which may be either unleashed or limited by the environment, presumably by chemical means.

But a later development has also been described. So long as cells proliferate around a small preformed piece of carrot tissue, they rarely, if ever, differentiate, however large the tissue mass grows. (The very rare formation of roots in this way is invariably due to an initial in the original explant.) But when cell colonies grow from free cells they may promptly and spontaneously organize, first to form normal roots. Once a cell colony produces a root apex, the continued growth of the organized tip suppresses the random proliferation

of the cell culture, and it will continue unabated so long as the liquid cultures are rotated around a horizontal shaft. However, if the organizing cell colonies are transferred to a stationary, semisolid, medium so that the roots can be geotrophically stimulated to grow down and the cultures may be exposed to air, then a shoot apex may organize at the opposite end of the axis to the new-formed root tip. Thus from free carrot cells, differentiated as phloem parenchyma, a plantlet forms. Interestingly enough, these early-formed plantlets arise from cell masses that show a strong resemblance to the first stages of embryonic development, and when shoot tip and root tip are recognizable, they so strongly resemble young embryos as to baffle belief that they had not indeed grown in embryo sac and ovule from a fertilized egg (Steward, Mapes, and Mears 1958). In other words, the environmental and culture conditions and the chemical stimulus furnished by the coconut milk medium have unleashed the totipotency which resided throughout, although previously suppressed, in the carrot storage parenchyma and in the cells of the explanted and cultured tissue (Steward, 1958).

Two things are impressive about the conditions which release that evident totipotency. First the cells were obtained free—free from the controls and restraints of the plant body and the tissue or organ in which they had formed; secondly, the cells were induced to grow and divide by the aid of the nutritive fluids which normally nourish young embryos.

These conditions combine to superimpose upon the behavior of the mature carrot cells a type of growth and behavior which is strongly reminiscent of development from a fertilized egg. In fact, in this context, one now sees the fertilized egg as a diploid cell which can divide, in a liquid medium which permits it to divide and in a free space in which it has relatively unrestricted room to divide! Thereafter, however, the derivative cells soon become subject to controls which enter in by virtue of their position in the growing organism. Thus the cellular organization is here seen to be, to an astonishing extent, totipotent, for the freely dividing cells function like zygotes and, on the contrary, what makes the zygote do what it does is not only its special "nature" or organization but its interaction with a characteristic "nurture" and environment.

This interplay of the cellular organization with the factors that control cell division and development is again one of the outstanding problems of plant physiology which is now being opened to experimental attack and to interpretation in terms of chemical stimuli.

A further development concerns the behavior of the cell nucleus during tissue and free-cell culture as investigated on carrot cultures (Mitra, Mapes, and Steward 1960) and later on cell cultures of *Haplopappus gracilis* (Nitra and Steward, 1961). *Haplopappus* is a plant with only four ($n = 2$) chromosomes in two pairs of morphologically distinct chromosomes. The following facts are now known. Carrot cells which grow around a performed tissue explant tend to retain their normal and stable nuclear condition ($2n = 18$). When carrot cells grow free, however, many variations occur. Multinucleate cells occur with high degrees of polyploidy. But, it is even more suggestive that somatic reduction also occurs and haploid nuclei are seen. Breakage and recombination of chromosomes, very reminiscent of the effects of radiation,

occur in response to the growth stimulus of the coconut milk medium. Again, in *Haplopappus* somatic pairing may be followed by clear evidence of chiasma formation at disjunction, with a strong indication that somatic crossing over may occur (see Fig. 4). Only in carrot cells has the complete cycle from carrot plant, to tissue culture, to free cells, to reorganized plantlet, to storage organ, and to flower been retraversed; and when this is done the reorganized plants have again all been diploids with 18 chromosomes (Mitra, Mapes, and Steward 1960).

Nevertheless, a new vista now opens for cell physiological study, for one sees that reduction division and meiosis, and the nuclear events usually associated with reproductive sites and centers as not unique to these situations for, when cells escape from the limitations of their normal tissue environment, these nuclear events may be induced by chemical means. Perhaps the coconut milk is not merely a cell division stimulant, but it may also function as a mutagen. The implications, therefore, for the understanding of development and the contrasted roles of the cellular organization, its nature and nurture, and for the translation into the behavior of somatic cells of some of the classical observations of cytology and genetics, which have been more familiar in relation to sex cells, are very great indeed. The implications for the study of development and morphogenesis are also obvious.

Again the gains are being, and will be, made by combining the experimental techniques of physiology and biochemistry with an appreciation of, and respect for, the organization of the cells and organism upon which they act.

Trends in Growth, Development, and Morphogenesis

Causal, or physiological, plant anatomy and experimental morphology have come to their present prominence in comparatively modern times, but while the foundations of plant physiology were being somewhat belatedly made (often awaiting needed developments in physics and chemistry) the great edifice of classical, embryological, morphological, and anatomical knowledge about plants was reared. Unsatisfied with the purely descriptive approach of morphologists, which too often was preoccupied with phyllogeny and evolution to the exclusion of developmental considerations, and preoccupied with their own experimental and biochemical tasks, physiologists drifted too far from morphology. This trend is now being, and should be, reversed as physiologists and morphologists meet in the stimulating field of experimental morphology.

It would take too long to trace the interpretation of growing points in the work of Sachs, of Hannstein, and later of Schuepp, and even in the more recent works of Foster, Clowes, and others. The relevant point, however, is simply and easily made. From their earliest inception in the zygote, the growing regions of shoot and root, by virtue more of their organization than of the inherent properties of their individual cells, grow in a highly controlled way to produce the great range of form and function which the organs of the plant body present. Yet, basically, all this wealth of morphogenetic development has to be built up as cells divide, grow, and differentiate. To enunciate rules of cell division; to recognize the controlling stimuli that operate in the growing regions of shoot and root; to determine the frequency and pattern

of cell division which causes growth to occur as it does; these are the ultimate goals of the study of development and morphogenesis. However, as shown above, a start has been made toward understanding the factors that determine the division of cells and with the recognition that free cells—more than those in preformed organized units—grow independently and then, like embryos, express their innate totipotency.

But the crux of the problem comes when one tries to interpret what happens in any shoot and root growing point and why, from the outset, their behavior is so distinct and polarized. It is true that many growing points of shoots can be greatly modified by etiolation (growth in complete darkness) or by growth submerged in water (which may, in some cases, induce growth which more nearly approaches that of a root). Whereas root apices usually appear first in embryogeny, they can often be regenerated on shoots (as in many familiar facts of propagation) but more rarely will a root give rise to shoots. Whereas attempts to culture angiosperm (especially dicotyledonous) root tips have often been crowned with success, even if the severed tip is small, the corresponding attempts to cultivate the shoot tip generally fail if the explant is so small that it includes only the central growing region without numerous leaf primordia and internodes from which it can also regenerate roots. Root and shoot may, therefore, be more metabolically interdependent than is understood.

Ideas upon the organization of growing points have, however, changed markedly. Views upon the actual distribution of cell division in the apices of shoot and root have changed with the recognition that there is often a quiescent region near the root tip in which central cells seem to enlarge early and do not, therefore, play a very active role in cell division. Furthermore, the main sites of division in the shoot apex are now seen to be much less at its surface than was hitherto thought and much more along its flanks, where leaves originate, with pronounced centers of meristematic activity in the so-called rib meristematic regions. Again in shoot apices, central and quiescent regions are being recognized. The physiological significance of the so-called quiescent regions can only be surmised, though they may well elaborate certain nutrients and stimuli which are supplied to the adjacent and more actively dividing cells. No longer is it tacitly assumed that the extreme apex or tip of shoot and root is of necessity a great center of metabolic activity, for Brown and his co-workers (Sunderland, Hayes, and Brown 1956) even conclude that the shoot tip is like an outer mantle of comparatively little activity surrounding a central core of metabolically more active cells.

Sufficient has been said, therefore, to show that physiologists interested in growth and development have made a start on the experimental attack upon the great questions which are raised by the way the growing points grow.

But the outstanding example of the great morphogenetic problems that need interpretation in terms of the physiological responses of apical growing regions are the problems of floral induction and the transition from vegetative to reproductive growth. It is not enough to describe, in the behavior of a normally short day plant (e.g. *Xanthium*), the effect of a single long night which will cause flowering to ensue, nor that the one long night may be interrupted by a single short flash of light to counteract that stimulus. Eventually

we shall need to know precisely how the stimulus of night, or the effect of interrupted light, affects the growing point itself. To date, most attempts to see visible effects upon the growing region of the shoot apex which are commensurate with the morphogenetic influence so imparted have been disappointing, although Wetmore's (1956) and Wetmore, Gifford, and Greene's (1959) account of the effect of photoperiodic stimuli upon the redistribution of cell division in certain shoot apices is a major step in the right direction. Clearly, however, the task will not be accomplished till one knows how photoperiod and thermal period, vernalization, rest period, and dormancy influence the actual growing regions. With confidence one can predict that, however the stimulus is "perceived", it will ultimately be transmitted to and affect the actual growing cells by some chemical means which still needs to be understood. Perhaps even as this is written the mechanism of "perception" of the photoperiodic stimulus is nearing elucidation by the well-known work of Hendricks and Borthwick (1954). Even so, however, there remains the problem how the effect is mediated upon the cells of the growing regions.

Trends in Molecular Biology

This was referred to in the preamble. It is, however, well to reiterate that certain limitations are evident in the current "molecular" approach to biology. This approach is essentially based on the views, largely elaborated from animal systems or bacteria, that the entire blueprint covering the fate of the organism through its life cycle is embodied in the information imprinted upon the DNA of the chromosomes, and that the extent of "coding" which is feasible by the sequence of bases along these polynucleotide chains can incorporate all the information necessary. This approach has met with conspicuous success in the interpretation of certain metabolic abnormalities in which specific genes cause the formation of unit changes in the structure of a given protein, as, for example, the well-known and remarkable work on inherited abnormalities in the structure of hemoglobins. Obviously such important contributions will remain part of the interpretation of living things and where such direct parallels can be drawn their meaning is clear.

However, one should also see in the complex interactions of gene-controlled factors in development the need for other controlling influences. A given cell in a given environment apparently endowed with the same totipotent genetic information may do very different things. It may be a quiescent phloem parenchyma cell and make stored nitrogen compounds and carotene or it may grow apace, synthesize a maximum of protein and even some protein of a characteristic kind which seems peculiar to proliferating cells, for it contains much hydroxyproline and is metabolically inert (Pollard and Steward 1959). Again it may behave like a zygote and form a cellular clump which will organize to form a root, then a shoot, and grow into an "embryo" plantlet and a whole plant. Examples may be multiplied indefinitely, but the lesson and the challenge is clear.

Additional to the genetically imparted information, which is carried in the structure of DNA and is implemented along the gene–enzyme–protein lines of conventional "molecular biology", we should see plant cells and the organism in even broader terms. This is so because the charted path of the organism,

for which the DNA is the blueprint, allows the organization great latitude in response. The initial trajectory or path of the organism, likened to that of a missile, charts the course in broad terms. But this may be modulated along the way, by photoperiod and night temperature, by etiolation or insolation, by the incidence of rest and dormancy, by vernalization, etc. Or again the information may be suppressed in the course of differentiation and development to permit otherwise totipotent cells to assume their specialized functions in the plant body. The evidence indicates that the cellular organization is responsive to a variety of exogenous chemical controls which modify its behavior in these respects. In part, these controls flow from factors determined by position in the growing regions of shoot and root; in part they flow from superimposed substances (whether conventionally called auxins, kinins, gibberellins, or chemical growth regulators) such as those which have been shown to be effective in morphogenetic experiments and in tissue culture.

These later effects, associated with development, may be likened to the instructions relayed from the bridge to the engine room, which modify, in accordance with prevailing conditions, the predetermined path of a vessel. Like the vessel, or the missile, the organism starts off with a thrust or "built-in" capacity to grow, mysteriously conveyed to the zygote at fertilization and fostered by the contents of embryo sac and of endosperm which provide its early nourishment. In part this capacity to grow works itself out, subject to metabolic fuel and nutrition, just as the missile, or ocean liner, consumes its fuel. But the "built-in" capacity to grow may be modified, modulated, and controlled by a variety of factors which are not genetic, not inherently "built-in". These "epigenetic" factors (cf. Waddington, 1957) seem to operate through the ability of the cell's organization to respond to a series of chemical controls, controls which affect its ability to grow and divide or to enlarge or to metabolize in a particular way.[2]

In the working out of these problems, which will require biochemistry to be combined with the intimate study of organization, developmental plant physiology has a bright and distinctive future amongst the biological sciences.

In modern science it is often felt that ultimate truths should be expressed in mathematical terms. Plant physiology and plant physiologists may indeed be, and have been, reproached for their comparative inability to do this. So long, however, as the mathematical approach can at best describe what happens after it happens (as in the analysis of growth curves), and especially

[2]None of the terms available (hormone, vitamin, auxin, kinin, gibberellin, caline) quite expresses the arrangements for control of the path taken during growth and development, for each has connotations peculiar to the circumstances in which it originated and implies some idea of the mechanism of the active agents. It has been drawn to my attention that no Greek word quite expresses the system or arrangements by which growth could be steered, controlled, or modulated. However, the Latin word "moderamen" was used for the arrangements by which the helmsman of a vessel, or a charioteer, determined or controlled its course. One might, therefore, speak of the whole system of controls in the cellular organization as the "moderamen", the various factors which modulate or control by interacting with the predetermined organization would be "moderaminants", without regard to how they act, and this type of interaction or effect could be "moderaminous". Terms are only valuable insofar as they clarify ideas and, as has been said, the nearest equivalent in use is the idea of "epigenetic" factors, a term which is only restricted by the implied connection with, or similarity to, the genetic apparatus.

where it fails to attach biological significance to the constants employed, this approach has not been very illuminating.

Therefore, whilst modern physicists can look, hopefully, to an all-embracing equation which expresses what one needs to know about space and time, matter and energy, this in biology is very far ahead indeed. The present complexities of the cells and organs which do grow and the many variables, nutritional, environmental, metabolic, genetic, etc., which affect growth, and the equilibrium and non-equilibrium processes involved in growth, would seem to require a mathematical interpretation of unworkable complexity to describe what a growing organism is. However, in due course it may be possible to put into a modern computing machine the analogues of the information that goes into the zygote and then predict how the organism, as it grows, will respond to external stimuli. Till this is feasible, we can progress by assessing on the one hand precisely what organized structures actually do, and, on the other, how they respond to chemical and environmental stimuli. This is surely what modern developmental plant physiology is about. Biochemistry and enzymology have gone far to explain the course of metabolism; genetics and molecular biology have gone far to show how the individual characteristics of the organism are predetermined; knowledge of inorganic and organic nutrition goes far to show how plants are nourished but the outstanding gap that needs to be closed is an adequate understanding of the organization of cells and growing regions which permits them to function as they do.

References

ANDERSON, D. B. and KERR, T. 1938. Growth and structure of cotton fiber. Ind. Eng. Chem. **30**, 48.

ROELEFSEN, P. A. 1959. The plant cell wall. *In* Encyclopedia of plant anatomy. *Edited by* K. Linsbauer. Gebruder, Borntrager, Berlin.

BENNET-CLARK, T. A. and KEFFORD, N. P. 1953. Chromatography of the growth substances in plant extracts. Nature, **171**, 645.

GAUTHERET, R. J. 1959. La culture des tissue vegetaux. Masson et Cie., Paris. p. 863.

HABERLANDT, G. 1902. Culturversuche mit isolierten Pflanzenzellen. Sitzber. Akad. Wiss. Wien, Math.-naturu. Kl. 111, Abt. I, 69–92.

——— 1921. Zur Physiologie der Zellteilung. 6, Mitteilung uber Auslosungvon Zellteilung durch Wundhormone. Sitzber. kgl. preuss. Akad. Wiss. 221–234.

HENDRICKS, S. B. and BORTHWICK, H. A. 1954. Photoresponsive growth. *In* Aspects of synthesis and order in growth. *Edited by* D. Rudnich. Princeton University Press, Princeton, N.J. pp. 149–167.

JONES, E. R. H., HENBEST, H. B., SMITH, G. F., and BENTLEY, J. A. 1952. 3-indolylacetonitrile: a naturally occurring plant growth hormone. Nature, **169**, 485.

LUCKWILL, L. C. 1959. Fruit growth in relation to internal and external chemical stimuli. *In* Cell, organism and milieu. *Edited by* D. Rudnick. Ronald Press Co., New York. pp. 223–251.

MITRA, J., MAPES, M. O., and STEWARD, F. C. 1960. Growth and organized development of cultured cells. IV. The behavior of the nucleus. Am. J. Botany, **47**, 357–368.

NITRA, J. and STEWARD, F. C. 1961. Growth induction in *Haplopappus gracilis*. II. The behavior of the nucleus. Am. J. Botany, **48**. In press.

NITSCH, J. P. 1955. Methods for the investigation of natural auxins and growth inhibitors. *In* Chemistry and mode of action of plant growth substances. *Edited by* R. L. Wain and F. Wightman. Butterworth Scientific Publications, London. pp. 3–31.

POLLARD, J. K. and STEWARD, F. C. 1959. The use of C^{14}-proline by growing cells: Its conversion to protein and to hydroxyproline. J. Exptl. Botany, **10**, 17–32.

POPE, D. T. and MUNGER, H. M. 1953*a*. Heredity and nutrition in relation to magnesium chlorosis in celery. Proc. Am. Soc. Hort. Sci. **53**, 472–480.

——— 1953*b*. The inheritance of susceptibility to boron deficiency in celery. Proc. Am. Soc. Hort. Sci. **61**, 481–486.

ROELOFSEN, P. A. 1959. The plant cell-wall. Gebruder Borntraeger, Berlin-Nikolassee.
SAGER, R. 1958. The architecture of the chloroplast in relation to its photosynthetic activities. Brookhaven Symposium in Biol. **11**, 101–117.
STEWARD, F. C. 1961. Organization and integration: some problems of cells, their growth and nutrition. Purdue Symposium on Growth.
——— 1958. III. Interpretation of the growth from free cells to carrot plant. Am. J. Botany, **45**, 710–713.
STEWARD, F. C., BIDWELL, R. G. S., and YEMM, E. W. 1956. Protein metabolism, respiration and growth. A synthesis of results from the use of C^{14}-labeled substrates and tissue cultures. Nature, **178**, 734–738, 789–792.
——— 1958. Nitrogen metabolism, respiration and growth of cultured plant tissue. III. Experiments with C^{14}-labeled substrates. Exptl. Botany, **9**, 37–51.
STEWARD, F. C., CRANE, F., MILLAR, F. K., ZACHARIUS, R. M., RABSON, R., and MARGOLIS, D. 1959. Nutritional and environmental effects on the nitrogen metabolism of plants. Symposia Soc. Exptl. Biol. **13**, 148–176.
STEWARD, F. C., MAPES, M. O., and MEARS, K. 1958. II. Organization in cultures grown from freely suspended cells. Am. J. Botany, **45**, 705–708.
STEWARD, F. C., MAPES, M. O., and SMITH, J. 1958. Growth and organized development of cultured cells. I. Growth and division of freely suspended cells. Am. J. Botany, **45**, 694–703.
STEWARD, F. C. and MOHAN RAM, H. Y. 1961. Determining factors in cell growth: Some implications for morphogenesis in plants. *In* Advances in morphogenesis. Vol. I. *Edited by* J. Brachet and M. Abercrombie. Academic Press, Inc., New York and London. pp. 190–265.
STEWARD, F. C. and MÜHLETHALER, K. 1953. The structure and development of the cell wall in the *Valoniaceae* as revealed by the electron microscope. Ann. Botany (N.S.), **17**, 293–316.
STEWARD, F. C. and SHANTZ, E. M. 1959. The chemical regulation of growth (some substances and extracts which induce growth and morphogenesis). Ann. Rev. Plant Physiol. **10**, 379–404.
STEWARD, F. C., SHANTZ, E. M., POLLARD, J. K., MAPES, M. O., and MITRA, J. 1961. Growth induction in explanted cells and tissues: metabolic and morphogenetic manifestations. *In* Growth Symposium. *Edited by* D. Rudnick.
STEWARD, F. C. and SUTCLIFFE, J. F. 1959. Plants in relation to inorganic salts. *In* Plant physiology – A treatise. Vol. II. *Edited by* F. C. Steward. Academic Press, Inc., New York. pp. 253–478.
STOWE, B. B. and YAMAKI, T. 1957. The history and physiological action of the gibberellins. Ann. Rev. Plant Physiol. **8**, 181–216.
SUNDERLAND, N., HAYES, J. K., and BROWN, R. 1956. *In* The growth of leaves. *Edited by* F. L. Milthorpe. Butterworth Scientific Publications, London. pp. 77–90.
WADDINGTON, C. H. 1957. The strategy of the genes: A discussion of some aspects of theoretical biology. The Macmillan Co., New York. p. 262.
WETMORE, R. H. 1956. Growth and development in the shoot system of plants. *In* Cellular mechanisms in differentiation and growth. *Edited by* D. Rudnick. Princeton University Press, Princeton, N.J. pp. 173–190.
WETMORE, R. H., GIFFORD, E. M., and GREENE, M. C. 1959. *In* Photoperiodism in plants and animals. *Edited by* R. B. Withrow. Am. Assoc. Advance. Sci. Publ. No. 45, pp. 255–373.
WHALEY, W. G., MOLLENHAUER, H. H., and KEPHART, J. E. 1959. Endoplasmatic reticulum and Golgi structures in maize root cells. J. Biophys. Biochem. Cytol. **5**, 501.
WHALEY, W. G., MOLLENHAUER, H. H., and LEECH, J. H. 1960. The ultrastructure of the meristematic cell. Am. J. Botany, **47**, 401–449.

ENDOGENOUS RHYTHMS AND MORPHOGENESIS[1]

E. BÜNNING

Physiologists have known for a long time that the presence of various distinct structures within a cell makes it possible for a number of different processes to proceed simultaneously without interference with one another. Less known, however, is the fact that a cell has also a mechanism which allows it to separate, in time, various processes which occur during its development. Thus, a cell can switch on and off two qualitatively different physiological states one after the other and thereby provide certain extreme physiological conditions in alternation. Some of the physiological processes are possible only in one of these extreme conditions.

The importance of such an alternation in the physiological states of a cell has been revealed from a number of recent experiments with synchronized cultures of microorganisms. A great deal is also known about the so-called endogenous diurnal rhythms of physiological conditions, which occur in the cells of multicellular organisms, that is, about the cyclical changes with a period of about 24 hours, which occur in cells existing even under constant external conditions.

A great deal of our knowledge of these approximately diurnal oscillations in plants and animals has been brought together in the Cold Spring Harbor Symposium on Biological Clocks held this year. It has been generally agreed to refer to these oscillations as "circadian rhythms" (*circa*, about; *diem*, day) and the importance of these circadian rhythms for plant and animal development has been extensively discussed.

Among the processes which are dependent upon the endogenous rhythm are, for example, photosynthesis and respiration. Thus there is in a cell an alternation between the two physiological states, one of which favors photosynthesis and the other respiration. One can also cite a number of other processes controlled by such a "physiological clock", like bioluminescence, growth, turgor, sporangial discharge, permeability, certain biochemical syntheses, and so on (1, 5).

The importance of this endogenous rhythm for morphogenesis becomes apparent from a number of observations. We know that this rhythm may disappear under constant conditions (in continuous light, for example). When this does happen, the alternating extreme physiological states are not attained and the cell can no longer carry out certain processes, which are dependent on the occurrence of these states (Fig. 1*a*). As a consequence, there arise physiological blocks. These blocks are manifested when plants grown in constant light show damage or the absence of certain developmental features. This is illustrated, for example, by the failure of the flowers of certain plants to

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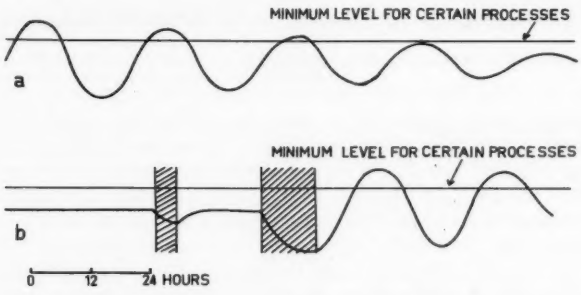

FIG. 1*a* and 1*b*. The role of endogenous rhythm in physiological processes.
FIG. 1*a*. Under constant conditions (continuous light or darkness at constant temperatures) the diurnal oscillations of the rhythm gradually die away. Physiological processes dependent upon different maxima in oscillations may finally cease.
FIG. 1*b*. A short dark period does not suffice to reinitiate diurnal oscillations after a long period of continuous light. A dark period of at least 8–10 hours is required. Such a dark period restores the processes blocked under constant light.

open under continuous light or by the failure of sporangia in several lower plants either to develop or to discharge their spores. Insofar as such blocks rest on the absence of diurnal oscillations, they are easily eliminated by the resumption of oscillations. In a constant light this may be brought about by the insertion of a single dark period of 8–10 hours' duration, as is shown in Fig. 1*b*. It may also be restored by a single exposure to low temperature (+5°, for example).

There are still other indications of the importance of endogenous rhythms to morphogenesis. Cell sensitivity to light and temperature both have been reported as characteristics regulated by the rhythm. This sensitivity, in fact, provides the basis for the thermo- and photo-periodic reactions (1, 11). Endogenous rhythms apparently cause an alternation between the two physiological states, which differ both quantitatively and qualitatively in their sensitivity to light and temperature.

In the following discussion I shall limit myself to the relationship between the endogenous rhythm and photoperiodic reaction, though other processes may also be referred to in order to clarify some particular detail.

It must be made clear at the beginning of this talk that photoperiodism is not, as is often assumed, a reaction related solely to floral initiation. A number of physiological processes in both plants and animals may be also photoperiodically controlled. Thus in some plant species, photoperiodic reactions are involved in the germination of seeds or in the induction and breaking dormancy of buds and bulbs. On the other hand, in other species these same phenomena may be independent of the length of the photoperiod. This diversity in reaction is similar to that observed in our operation of many technical processes. Thus industrial machines may be turned on and off either directly by hand or by means of a time-clock.

Photoperiodic control of a physiological process means that, as shown in Fig. 2, one of the intermediate stages of such a process contains a built-in

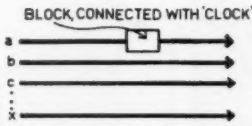

FIG. 2. Photoperiodic control of physiological processes in plants and animals. Several parallel processes, indicated by the arrows, are necessary to enable a complex process like flower initiation, induction or breaking of dormancy to be completed. Some of these parallel processes may contain a block which can be lifted only by a time-measuring process controlled by the light–dark regime.

physiological block, which can be removed only by the right day length (i.e. by means of a time-measuring device). We are not concerned here, however, with the totality of the various stages involved in flowering as an example of a photoperiodic response, but only with the question of how such a block can be removed by a physiological time measurement.

The basis of photoperiodic time measurement is the existence of a critical day length. The plant can depend upon this critical day length to determine the right time of the year for carrying out its developmental processes. To be useful for such a purpose, the critical day length should be relatively independent of temperature and this is indeed the case.

I am not talking now about all the different temperature effects either on flowering or on other processes which can be under control of the day length. I am talking only about the temperature effect on the critical day length. Direct temperature effects on all other processes represent quite a different matter.

This low temperature coefficient has been the stumbling block in the way of previous attempts to explain the critical day length in terms of a single biochemical reaction. It had been thought that the beginning of either light or dark periods initiated a single biochemical reaction, which was completed only after a definite time (the critical day length) had elapsed. One would expect that the rate of such a reaction would be strongly influenced by the temperature.

Now we have a clear proof that the time measurement in photoperiodic reactions is accomplished, not by a single biochemical process in the classical sense, but with the help of the endogenous rhythm. In all the cases studied so far, the length of the periods in this rhythmicity is known to be rather temperature independent (1, 5). Thus the suggestion of the endogenous rhythm for the timing in photoperiodism explains the relative temperature-independence of the time-measuring process. In illustrating this relationship we shall limit ourselves to the short-day plants. The basic considerations in such an explanation are indicated in Fig. 3, *a*, *b*, and *c*.

We see in Fig. 3*a* that there is an upper limit to the length of day under which a short-day plant can flower. This limit is called the critical day length. According to the "classical" hypothesis, the critical length for the light (or dark) period is determined by the time required for the completion of one or two processes, as shown in Fig. 3*b*. However, it may be easily demonstrated that so-called light or dark processes are correlated with the endogenous

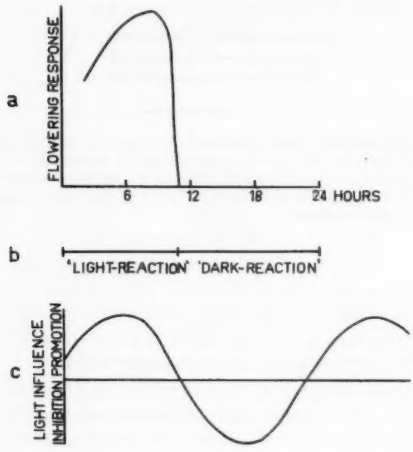

FIG. 3*a*, 3*b*, and 3*c*. The role of endogenous rhythm on the induction of flowering in a short day plant.

FIG. 3*a*. Relationship between the day length and floral initiation. Abscissa: length of light period.

FIG. 3*b*. The "classical" explanation of the relationship indicated in Fig. 3*a*. One or two of the processes must occur, requiring (in this example) 11 and 13 hours for the induction.

FIG. 3*c*. Explanation of the time measurement in terms of the endogenous rhythm. The rhythm causes an alternation between one state, in which light promotes flowering (photophil phase), and another, in which flowering is inhibited by light (scotophil phase).

rhythm of the plant. That is, the rhythm illustrated in Fig. 3*c* causes an alternation between the two physiological states, which differ qualitatively in their response to light. Naturally, these cycles of light sensitivity, i.e. changes in the quantity and quality of the response to equal light stimuli offered at different times, are to be regarded as a consequence of the rhythm and not as the clock itself.

The correctness of this interpretation may be demonstrated in several plants which show other rhythm-controlled phenomena occurring concurrently with that of the photoperiodic flower induction. These other phenomena may serve as the "clock hands" to indicate the clock's progress. Thus simultaneously with the observations on floral induction, one can also follow the course of this internal clock, by observing plant responses to light stimuli given at various times.

Let us observe such a relationship in a single plant *Kalanchöe blossfeldiana*. This plant shows, besides rhythmic light sensitivity, also another easily measurable process controlled by its internal clock. For the "clock hands" we may use the opening and closing movements of its flowers. For such observations we must use, of course, plants already in bloom, while non-induced plants will serve for the determination of their photoperiodic floral induction. It has been shown in numerous experiments by Bünsow (4) that opening of the flowers corresponds to the physiological state of a plant in

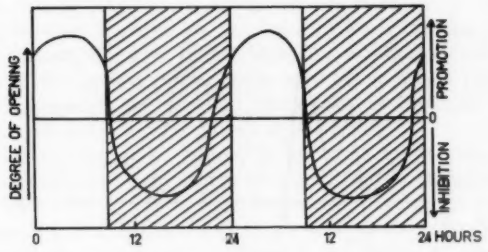

FIG. 4. Relationship between the opening and closing movements of petals and the daily rhythmic changes of photoperiodic light sensitivity in *Kalanchöe blossfeldiana* grown in light–dark cycles of 9/15 hours.
Left ordinate: relative aperture of flowers.
Right ordinate: action of light on flower initiation (data taken from Bünsow).

which light promotes floral initiation (photophil phase), while closing corresponds to that in which light inhibits flowering (scotophil phase). This relationship is illustrated in Fig. 4.

We shall now consider a few examples of the numerous published and unpublished experiments carried out on this subject by several workers in this field.

As is illustrated in Fig. 5, when a plant is grown on a normal light–dark cycle (9 hours of light and 15 hours of darkness) introduction of light flashes during the dark periods inhibits flowering, the extent of inhibition being dependent upon the time at which the flash is given (8). The same figure also gives the movement of petals, as observed in comparable plants grown on the same light–dark cycle, but without light flashes. Such petal movements serve as a "clock hand" of the rhythm and the synchronization of both curves in this figure is apparent.

As is shown in Fig. 6, the diurnal rhythm continues even in plants which were placed on 8 cycles with very long dark periods (10 hours of light and 62 hours of darkness). Prior to and after these cycles, plants were grown on con-

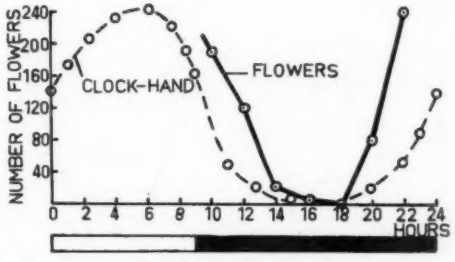

FIG. 5. Relation between the initiation of flowers and the movement of petals in *Kalanchöe blossfeldiana*.
"Clock hand" curve after Harder and Bode (7). The graph for the floral initiation from our own laboratory. White light flashes are of 1-minute duration.

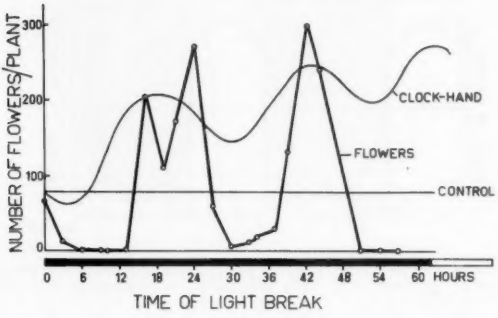

FIG. 6. Effect of a long dark period on the initiation of flowers in *Kalanchöe blossfeldiana*.

tinuous light. Red light flashes were of 2 minutes' duration (maximum at 650 mμ, each flash had 660 kiloergs × cm^{-2}) and these were given at various times in the long dark period. Continuation of the diurnal rhythm during the long dark period is apparent from the petal movement ("clock hand"), observed on plants kept under comparable conditions (3, 9). A physiological state (a photophil phase), in which light promotes flowering, continues to alternate during this long dark period with another state (scotophil phase) in which light is inhibitory. Each of these alternating phases lasted about 12 hours.

It was shown by Hamner and his co-workers in their experiments with soyabean (7) that an increase in the length of dark period did not always result in the stimulation of floral induction. The results of our own experiments with *Kalanchöe*, presented in Fig. 7, reveal that a further increase in a dark period beyond 18 hours actually gives a lower induction than a dark period of only 18 hours. Minimal induction is reached with a dark period of 24–27 hours. However, a second period of maximal induction is obtained with a dark period of 42–48 hours, while a second minimal period occurs with a dark period of 66–72 hours.

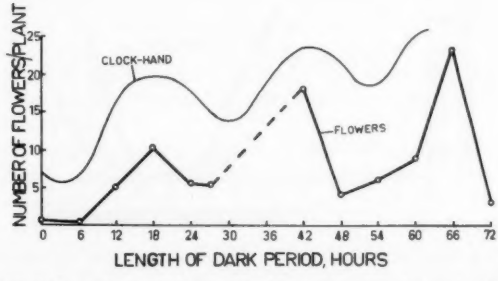

FIG. 7. Effect of the extension of the dark period on flowering and petal movements in *Kalanchöe blossfeldiana*. Both curves after Bünning and Engelmann.

These results can be explained, when we compare them with the graph showing the movement of petals as the "clock hands" of the internal clock. This last graph was obtained on plants kept in continuous darkness.

An extension of the period of darkness may occur either in the photophil or in a scotophil phase of the internal clock and its effect on flowering will depend in which one of these two phases it has fallen.

From the brief review given above it becomes quite clear that diurnal alternations in the physiological states of a cell, that is endogenous rhythms, play an important role in plant morphogenesis. Examples have been presented for *Kalanchöe*. For further details, as well as for information on other species, one should examine literature cited in the course of this presentation.

References

1. BÜNNING, E. 1958. Die Physiologische Uhr. Springer-Verlag, Berlin.
2. BÜNNING, E. 1960. Circadian rhythms and the time measurement in photoperiodism. Cold Spring Harbor Symposia Quant. Biol. Vol. XXV. Biological Clocks. In press.
3. BÜNNING, E. u. ENGELMANN, W. 1960. Endogen-tagesperiodische Schwankungen der photoperiodischen Hellrot-Empfindlichkeit bei *Kalanchöe blossfeldiana*. Naturwissenschaften, **47**, 332.
4. BÜNSOW, R. 1960. Photoperiodism and circadian rhythm in *Kalanchöe*. Cold Spring Harbor Symposia Quant. Biol. Vol. XXV. Biological Clocks. In press.
5. COLD SPRING HARBOR SYMPOSIA Quant. Biol. 1960. Vol. XXV. Biological Clocks. In press.
6. ENGELMANN, W. 1960. Endogene Rhythmik und photoperiodische Blühinduktion bei *Kalanchöe*. Planta, **55**, 496–511.
7. HAMNER, K. G. 1960. Photoperiodism and circadian rhythms. Cold Spring Harbor Symposia Quant. Biol. Vol. XXV. Biological Clocks. In press.
8. HARDER, R. u. BODE, O. 1943. Über die Wirkung von Zwischenbelichtungen während der Dunkelperioden auf das Blühen, die Verlaubung und die Blattsukkulenz bei der Kurztagpflanze *Kalanchöe blossfeldiana*. Planta, **33**, 469–504.
9. MELCHERS, G. 1956. Die Beteiligung der endonomen Tagesrhythmik am Zustandekommen der photoperiodischen Reaktion der Kurztagpflanze *Kalanchöe blossfeldiana*. Z. Naturforsch. **11b**, 544–548.
10. SCHWEMMLE, B. 1960. Thermoperiodic effects and circadian rhythms in flowering of plants. Cold Spring Harbor Symposia Quant. Biol. Vol. XXV. Biological Clocks. In press.
11. WENT, F. W. 1960. Photo- and thermoperiodic effects in plant growth. Cold Spring Harbor Symposia Quant. Biol. Vol. XXV. Biological Clocks. In press.

STRUCTURE AND COMPOSITION OF PLANT-CELL ORGANELLES IN RELATION TO GROWTH AND DEVELOPMENT[1,2]

GEORGE SETTERFIELD

Introduction

The complex processes involved in growth and development of plants may be studied at many levels and from many points of view. One main approach has been observation with the light microscope where the general form of the cell is revealed and some information on cell contents is obtained. This type of study has produced an extensive literature on plant cytology and anatomy (17) but a wide gap remains between such knowledge and the available information on biochemical and physiological processes occurring in growing tissues (62). Clearly one prerequisite for understanding the nature of growth and development is at least a partial bridging of this gap.

A number of new cytological and cytochemical methods such as electron microscopy, cell fractionation, autoradiography, and cytospectrophotometry have begun to provide information in this area between gross cellular structure and physiological processes. Most of such information is derived from animals but where studies have been performed on plant cells they indicate that structure and function at this level is basically similar in all higher organisms. Recently we have applied a number of these techniques to plant cells in both dormant embryos and growing tissues and this paper will summarize some of the results and relate them to similar information obtained by other workers.

The discussion will be organized around the three general areas of the plant cell: the nucleus, cytoplasm, and cell wall. The nucleus is, of course, the seat of genetic information, probably mainly in its DNA component, and is primarily of importance in supplying the plan for development. Expression of this genetic plan, on the other hand, undoubtedly occurs principally in the cytoplasm through control of the mechanisms of protein synthesis (10). One of the main problems of developmental physiology is elucidation of the means by which this control is achieved. Finally, in plant cells the wall represents the most obvious differentiating structure and is of importance as one of the main "end products" of cellular development (52). Specifically, cell wall formation raises the problem of how organized structure is produced through metabolic activity of the cytoplasm. Unfortunately, it is symptomatic of the fragmentary knowledge of all of these subjects that no over-all integrated discussion is possible and each area must at present be considered more or less independently.

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[2]The following abbreviations are used: DOC, desoxycholate; DNA, desoxyribonucleic acid; DNAase, desoxyribonuclease; ER, endoplasmic reticulum; RNA, ribonucleic acid; RNAase, ribonuclease.

The Nucleus

Fine Structure

It is a striking fact that while the major advances in cytology resulting from use of the light microscope concerned nuclear structure, electron microscope studies on nuclear organization have been relatively unrewarding (6, 59). No general functional interpretation of the observed fine structure within nuclei is yet possible so that this section will merely indicate some of the main structural features present.

The most consistent and best described nuclear structure is the envelope. In a number of organisms it has been shown to consist of two distinct parallel membranes 200–300 Å apart, which are regularly interrupted by pores about 400–1000 Å in diameter (45, 59, 71, 74) (Fig. 1). The double membrane aspect of the nuclear envelope is similar to the membrane systems of the cytoplasm referred to as endoplasmic reticulum (discussed below), and Porter and Machado (45) have recently shown that in dividing cells of onion root tips the nuclear envelope is indeed formed during telophase through coalescence of elements of the reticulum. Whaley and co-workers (72, 74) have also shown in meristematic cells of corn roots that the nuclear envelope shows numerous direct connections to the endoplasmic reticulum. It is thus possible, as suggested by Watson (71), to regard the nuclear envelope primarily as a specialized form of endoplasmic reticulum and therefore a structure basically of cytoplasmic origin (74). In keeping with this concept it may be noted that organisms lacking a typical membrane-bounded nucleus, such as bacteria and blue-green algae, also lack demonstrable endoplasmic reticulum in the cytoplasm.

The structural situation within the nucleus is much less clear. At low magnification dense areas probably representing chromatin are usually visible and between these are less dense interchromatic regions (59). The amount of dense chromatin varies with the DNA content of the nuclei. For example, little chromatin is apparent in nuclei of cucumber root (Fig. 1), while pea-embryo nuclei show prominent chromatin bodies (Fig. 2). Cytophotometric measurement of Feulgen stain indicates that cucumber nuclei contain only about 10% as much DNA as pea nuclei (57). Swift (59) has shown a direct correspondence between areas staining densely with Feulgen and the

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NOTE: All figures in Plates I, II, III, and V are electron micrographs.

SYMBOLS USED:

A.	Additional longitudinal microfibrils.	N.	Nucleus.
C.	Chromatin region.	Nm.	Nuclear membrane.
Cy.	Cytoplasm.	Nu.	Nucleolus.
D.	Dictyosome.	P.	Proplastid.
ER.	Endoplasmic reticulum.	R.	Ribosomes.
I.	Interchromatin region.	T.	Tonoplast.
Ic.	Intercellular region.	V.	Vacuole.
M.	Mitochondrion.	Ve.	Vesicular membranes.
		W.	Cell wall.

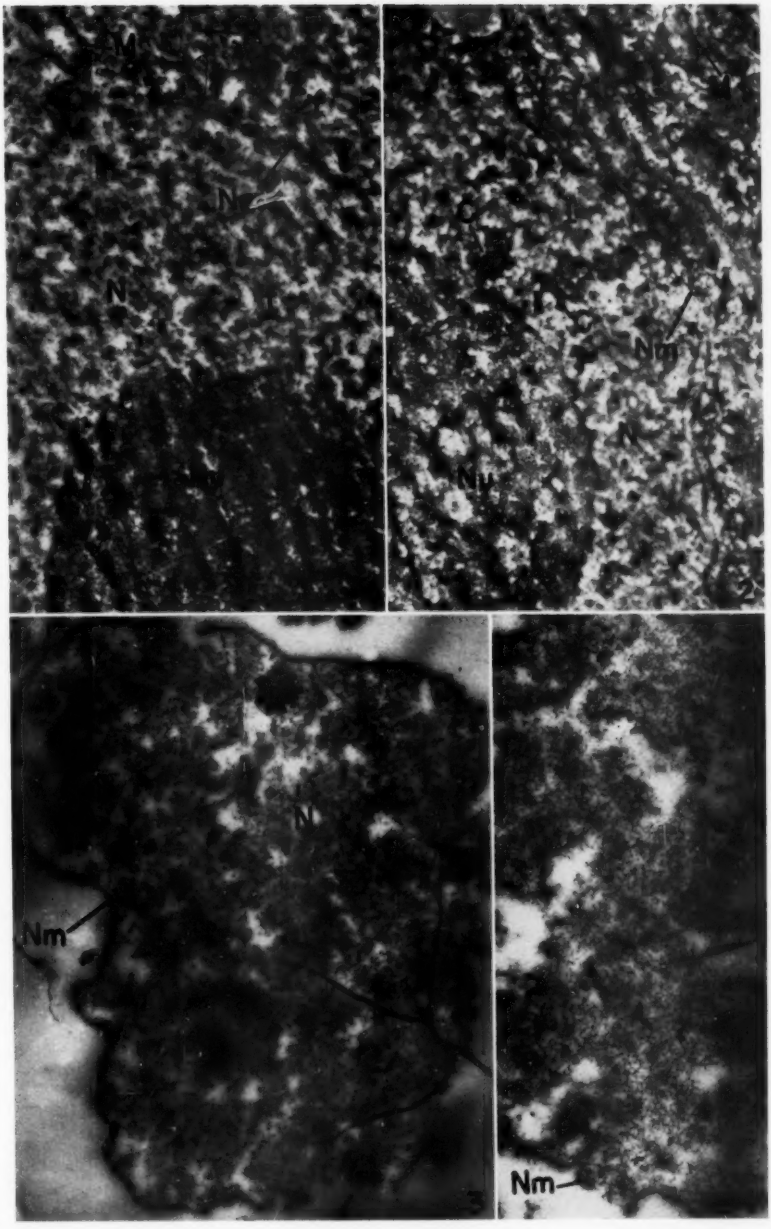
FIG. 1. Portion of nucleus and nucleolus in cucumber-root cell. × 28,500

FIG. 2. Portion of nucleus and nucleolus in pea-embryo cell. × 19,000

FIG. 3. Low magnification view of isolated pea-embryo nucleus. Stained in lead hydroxide. The dense black granules are precipitate formed during staining. × 11,500

FIG. 4. Higher magnification view of a pea-embryo nucleus as in Fig. 3. Note the delicate particulate component. × 18,000

PLATE I



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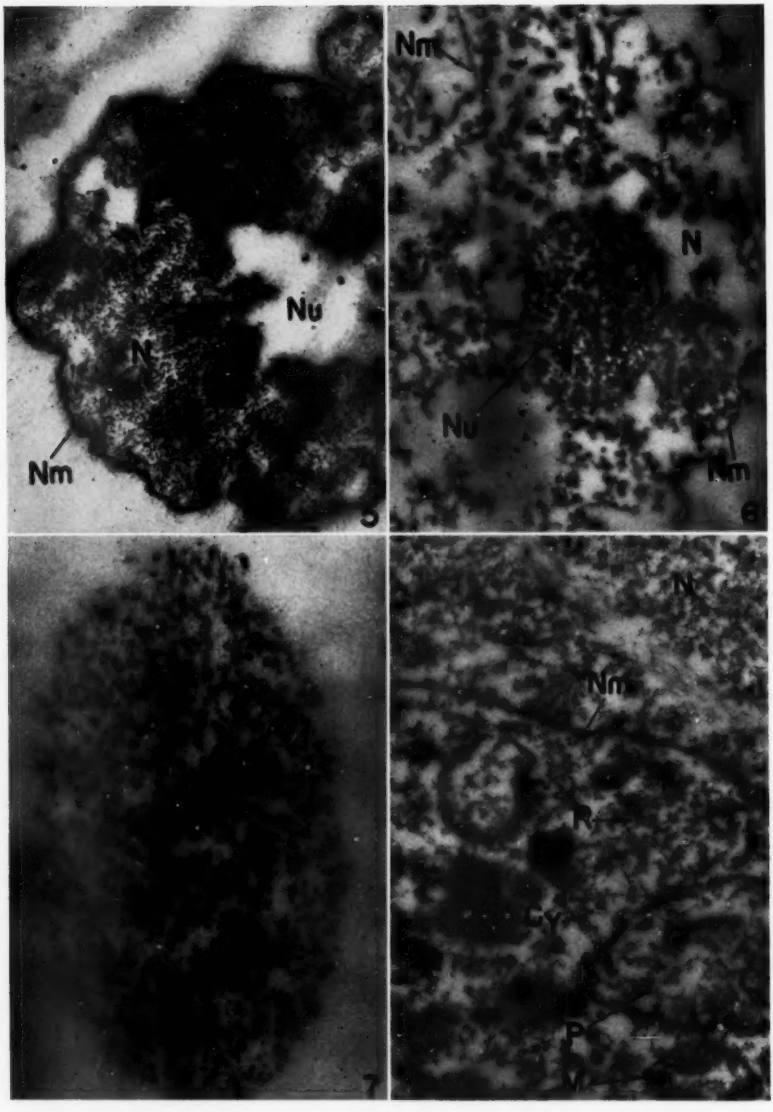

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chromatin areas visible in the electron microscope, although there is also staining between the chromatin regions.

Concerning fine structure, in thin sections of osmic-acid-fixed preparations of pea-embryo cells both chromatin and interchromatin regions of the nucleus appear to contain very irregular fibrillar and particulate material (Figs. 1, 2) but we are unable to recognize any very regular organization. Such lack of definitive organization at the level of nuclear fine structure is apparent in published electron micrographs of other authors (30, 59). In animal nuclei some definite granules, with which RNA possibly is associated, have been described (59) and a wide range of ribonucleoprotein particulate material has been isolated (18), but as yet no generally consistent picture of the fine organization has emerged.

Dr. S. T. Bayley and I have begun some studies on structural components of pea-embryo nuclei which were isolated by cell fractionation procedures (28). Figure 3 shows a typical section of such a nucleus fixed with osmic acid and stained in lead hydroxide, which has an affinity for structures containing nucleic acids (70). Isolation of the nucleus has disrupted the clear-cut separation into chromatin and interchromatin regions but cytophotometric measurements (57) indicate that little or none of the DNA is lost during isolation of the nuclei and apparently the dense chromatin regions have merely dispersed. The most obvious component of these nuclei is fine particulate material, which is increased in density by the lead staining, appearing as aggregations of granules or fibrils about 100 Å in diameter (Fig. 4). Moses and Lafontaine (33) have also suggested that such a fine fibrillar component is a basic feature of interphasic plant nuclei. The particulate material is essentially unaffected by treatment with RNAase prior to fixation (Fig. 5) but, on the other hand, is entirely destroyed in nuclei treated with DNAase before fixation in osmic acid (Fig. 6). It is thus likely that the DNA of these nuclei is associated with the fine particulate material which stains readily with lead. Unfortunately, the rather irregular nature of this material gives little insight into the over-all structural organization or the function of the interphasic chromosome. The lack of influence on structure by RNase treatment suggests that an obvious structural component containing RNA is not present in these nuclei, which, in turn, may reflect the fact that they are derived from metabolically inert tissue or perhaps loss of an RNA component during isolation.

Apart from the chromosomal region of the nucleus, the nucleolus is a ubiquitous nuclear feature which has long intrigued cell physiologists. Sirlin (55) recently discussed modern thinking about this structure and elaborated the two main theories concerning its function. Briefly, the nucleolus has been

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FIG. 5. Isolated pea-embryo nucleus treated with RNAase prior to fixation. Stained in lead hydroxide. Note the empty space where the nucleolus existed. × 15,800

FIG. 6. Isolated pea-embyro nucleus treated with DNAase prior to fixation. Note the absence of any fine particulate material in the nuclear region. × 11,000

FIG. 7. Isolated pea-embryo nucleolus. × 28,000

FIG. 8. Portion of a pea-embyro cell at the region of the nuclear membrane. Stained in lead hydroxide to reveal ribosomes. × 21,000

considered as (*a*) a central structure in the general functioning of the nucleus, or (*b*) a specialized structure concerned with the operation of one or a few genes. There seems little doubt that nucleolar formation is directly related to specific genetic loci (60), but at the moment it is impossible to decide how generally important in cell metabolism this structure is. Autoradiographic studies with both animal (1, 21) and plant (75) tissues suggest that the nucleolus may be a region where RNA is synthesized or accumulated prior to transport to the cytoplasm. It has also been proposed that nucleoli are regions of protein synthesis and that the proteins formed may be involved in cytoplasmic metabolism (55, 60). In addition, Seed (48) has shown that specific irradiation of the nucleolus with soft X rays interfered with nucleic acid synthesis in the nucleus. Such considerations all imply that the nucleolus is an organelle of basic importance in cell metabolism, particularly in the chain of reactions concerning nuclear control of cell processes.

Unfortunately, structural studies add little to these concepts of nucleolar function. The nucleoli of broad-bean and onion roots have been extensively studied in the electron microscope by Lafontaine (30) and appear similar to those we have observed in pea-embryo (28, 54) and cucumber cells (Figs. 1, 2). At low magnification the nucleolus is usually fairly homogeneous and of about the same density as the chromatin regions of the nucleus. At higher magnification it is sometimes possible to distinguish particulate elements within the nucleolus but their actual nature is unclear. Lafontaine (30) suggested that they may represent sections of threads or tubes but it is difficult to infer the three-dimensional organization from the electron micrographs of thin sections. Swift (60) has described a variety of structural elements in animal nucleoli and some of the ribonucleoprotein particulates isolated by Frenester *et al.* (18) from nuclei may be derived from nucleoli, but on the whole the general picture of nucleolar organization is obscure.

Nucleoli do not possess a bounding membrane and when isolated from pea-embryo nuclei by fractionation procedures they expand and reveal their internal structure somewhat more clearly (28). We have studied such nucleoli in the electron microscope and find a range of irregular profiles without any obvious organization (Fig. 7). It may merely be said that at least one component of plant nucleoli is a complex meshwork of fine particulate material as suggested by Lafontaine (30). Incidentally, although these isolated nucleoli appear to have lost some structural material as compared with in situ nucleoli (28) they have about the same RNA content as in situ nucleoli, as determined by cytophotometric measurement of azure-B staining at pH 4.0 (57).

Another interesting aspect of nucleolar structure emerged from isolated pea-embryo nuclei which were treated with RNAase prior to fixation (Fig. 5). In such nuclei the nucleoli were entirely removed despite the fact that RNA forms only about 10% of nucleolar composition (56). This result is quite distinct from those obtained when RNAase is applied after fixation (60), where nucleolar structure remains largely intact, and suggests that, in the native nucleolus, RNA is an essential, integral part of the nucleolar organization which on removal causes the preponderant protein to disperse or at least become unstable to osmic acid fixation.

One final negative result of our studies on nuclear structure should be

mentioned. Bonner (10) and other authors have suggested that ribosomes (ribonucleoprotein or microsomal particles), which are active agents in protein synthesis in cytoplasm (discussed below), are formed directly in the nucleus by gene action and moved to the cytoplasm. In our observations on pea-embryo nuclei we have been unable to see particles similar to ribosomes within either nucleus or nucleolus (Figs. 2, 8) and as noted above, the structure of the nucleus was unaffected by RNAase treatment. Furthermore, when we analyzed a homogenate of pea-embryo nuclei in the analytical ultracentrifuge we obtained only a small peak with a sedimentation coefficient of about 80 *s*, typical of ribosomes, which could easily be accounted for by known cytoplasmic contamination of the nuclear preparation. Thus, these embryo nuclei do not lend support to the hypothesis that ribosomes are formed in the nucleus. However, it must be admitted that these are results from nuclei of inert cells and may be atypical. Certainly in nuclei, such as that shown in Fig. 1, it is possible to see particles in the nucleus which might represent ribosomes "in transit" to the cytoplasm.

DNA Metabolism in Relation to Growth

It is impossible to discuss all aspects of nuclear metabolism here and attention will be directed to only one process, DNA synthesis. It is now well established that the amount of DNA within individual nuclei of the same organism closely parallels the number of chromosomes, or chromatids, present. For example, prior to chromosome replication diploid nuclei contain a basic amount of DNA designated in relative terms as 2C. If such nuclei are to divide, during the interphase preceding mitosis all chromosomes are duplicated so as to contain two chromatids each and the DNA content doubles, giving a relative value of 4C. During division each new cell then receives half of the chromatids and the DNA value of each becomes 2C (42). Diploid nuclei may thus contain either 2C or 4C amounts of DNA and similarly tetraploid nuclei may have 4C or 8C and octaploid nuclei 8C or 16C, etc. The actual process of DNA synthesis appears to be very rapid since very few nuclei showing amounts of DNA intermediate between the geometric C class values are encountered (58).

Apart from this simple duplication of DNA in relation to the mitotic cycle, several studies have suggested an association between DNA synthesis and mechanisms regulating growth in plants. Naylor (36) showed that nuclei in the apex of dormant lateral buds of *Tradescantia* contain the 2C amount of DNA and that stimulation of growth of such buds by removal of the apical bud is preceded by doubling of DNA to the 4C level. Napthalene acetic acid applied in place of the apical bud prevented DNA duplication. Patau *et al.* (41), working with excised tobacco pith, obtained evidence that auxin and kinin are both involved in DNA synthesis. The results of Naylor and of Patau *et al.* depended on cytochemical analyses but recently Biswas and Sen (8) have reported that auxin can stimulate incorporation of P^{32} into DNA of coleoptile segments.

Such results led us to investigate the DNA content of individual nuclei within growing oat coleoptiles. Figures 9 and 10 are multiple frequency diagrams which show the results from measurement of DNA in individual

nuclei of coleoptiles of different ages by the two-wave-length cytophotometric method, using Feulgen stain. Each individual histogram represents a random sample of nuclei from a single coleoptile. The histogram at the upper left in Fig. 10 shows DNA values from cell divisions, which serve as standards to indicate the class means: anaphases and telophases are by definition 2C and prophases and metaphases 4C (42). Figure 9 shows the situation regarding DNA contents of nuclei in coleoptiles of germinating embryos. In the ungerminated coleoptile (0 hour) essentially all of the nuclei are at the 2C level and this situation persists during the first 12 hours of germination. At some point during the second 12 hours of germination DNA synthesis begins and by 24 hours after the start of germination about half of the nuclei have reached the 4C level of DNA. Cell division also starts during the second 12 hours and by 24 hours an approximate steady state is apparently established in

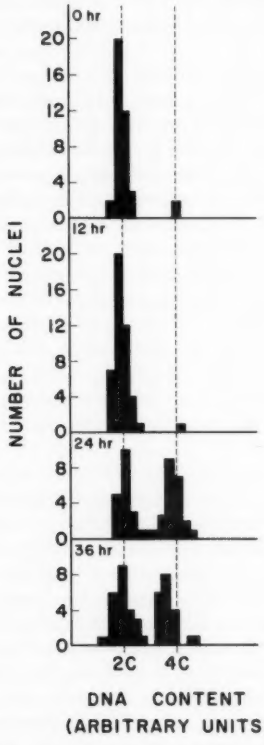

FIG. 9. Frequency diagrams showing relative DNA contents (Feulgen) of random samples of interphase nuclei in oat coleoptiles of germinating embryos. Times in hours indicate the periods the seeds were allowed to imbibe water. Obtained through cytophotometric measurements in 12 μ sections of tissue fixed in ethanol/acetic acid, 3/1.

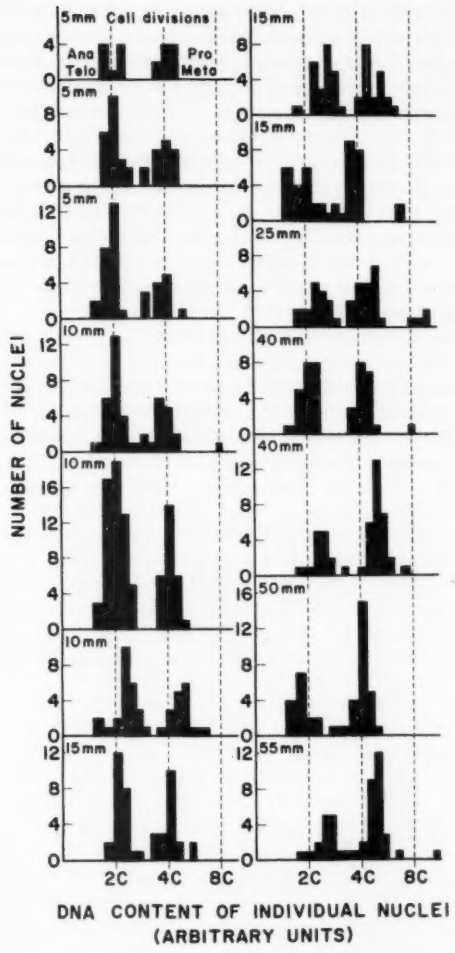

FIG. 10. Frequency diagrams showing relative DNA contents (Feulgen) of random samples of interphase nuclei in oat coleoptiles. Each individual diagram from a different coleoptile. Lengths of coleoptiles given in millimeters. The diagram at the upper left gives DNA contents of dividing cells to serve as a standard for the class means. In some preparations the class means were elevated for some unknown reason. Obtained through cytophotometric measurements in squashes of tissue fixed in ethanol/acetic acid, 3/1.

which the number of nuclei doubling their DNA is balanced by the number halving their DNA through division. This situation persists through 36 hours but as growth proceeds (Fig. 10) it is apparent that the rate of mitosis slightly exceeds that of DNA synthesis and there is a slow increase in the relative number of 2C nuclei. Cell division only continues until the coleoptile reaches a length of about 10 mm (owing to variability in rates of growth after the early stages of germination the histograms in Fig. 10 are related to length of the coleoptile rather than age in time) where about two thirds of the nuclei are 2C and one third 4C. However, although cell division ceases at this stage and growth continues solely by cell elongation, it is clear that DNA synthesis is not blocked and that individual 2C nuclei continue to double their DNA until at the cessation of growth (50-55 mm) the nuclei are predominately 4C. This slow, steady increase in 4C nuclei would explain the incorporation of P^{32} observed by Biswas and Sen (8) in elongating coleoptiles. The general picture is also quite similar to that inferred by Jensen *et al.* (27) from autoradiographic studies on onion roots and by Swift (58) from photometric determinations in the differentiating regions of roots.

Several interesting conclusions derive from these data on DNA in coleoptiles.

(1) It appears that DNA content is not directly related to the processes of cell expansion since at all stages of elongation cells containing both 2C and 4C nuclei were present.

(2) As in lateral buds of *Tradescantia* (36) the nuclei of the dormant embryonic coleoptile are held at the 2C level suggesting a fundamental mechanism involved in cell dormancy could be blockage of DNA synthesis and this might be related to growth control through plant hormones (Naylor, personal communication). However, all dormant plant nuclei are not necessarily held at the 2C DNA level since dormant pea embryos contain approximately equal amounts of 2C, 4C, 8C, and 16C nuclei (Fig. 11). Swift (58) also noted higher DNA classes in embryonic tissues. This situation needs

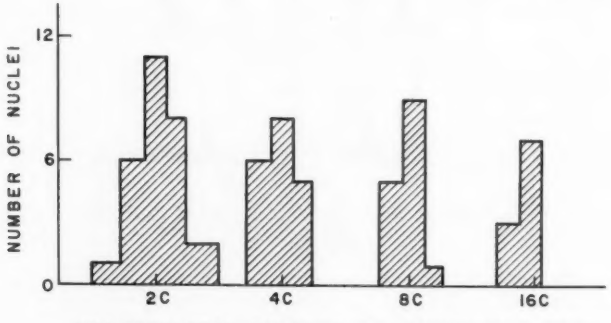

FIG. 11. Frequency diagram showing relative DNA contents (Feulgen) of a random sample of nuclei in an ungerminated pea embryo. Obtained through cytophotometric measurements in a squash preparation fixed in ethanol/acetic acid, 3/1.

further investigation since it is possible that only the 2C nuclei of the embryo contribute to further growth and the higher class nuclei are polyploid and are unable to double their DNA and divide when germination proceeds.

(3) The natural cessation of cell division in the 10-mm growing coleoptile appears metabolically quite distinct from that in dormant embryos. While DNA synthesis is blocked in the dormant tissue the inhibition of division in elongating cells appears to be through processes involved in entrance into mitosis and is independent of DNA synthesis. In fact it appears that doubling of DNA to the 4C level simply continues as if cell division were occurring. There would thus seem to be possibly at least two quite distinct mechanisms for organized control of cell division in plants: inhibition of DNA synthesis as in embryonic coleoptiles and lateral buds, and a prevention of entrance into mitosis independent of DNA level as in growing coleoptiles.

(4) Although throughout the growth of the coleoptile there is continuous doubling of DNA from 2C to 4C levels there seems almost a complete block on further doubling to higher class values. The few 8C nuclei present were in epidermal cells and were present in fairly young coleoptiles. This situation is somewhat distinct from other plant tissues such as pith (41) and roots (58) where cells regularly become polyploid. The significance of such differences in degree of polyploidy in different tissues remains unknown but at the moment there does not appear to be an obvious relationship with growth and differentiation.

Cytoplasm

Fine Structure

Probably the most rewarding use of the electron microscope in cytology has come through studies on the cytoplasm of higher organisms. As a result of intensive investigation for almost a decade, the fine structure of a number of cytoplasmic organelles has been thoroughly elucidated and the general similarity of this structure has been shown in a wide variety of organisms from both animal and plant kingdoms. In higher plants attention has largely been directed to meristematic cells, which are readily preserved for microscopic examination, and Whaley *et al.* (73) have recently reviewed the extensive literature on cytoplasmic structure in these cells. The main cytoplasmic organelles of such cells may be described briefly as follows.

(1) Proplastids. Both roots (73) and etiolated shoot (Fig. 14) tissues commonly show proplastids. These are relatively large globular bodies bounded by double membranes and containing various types of internal membranes embedded in a rather structureless matrix. Proplastids are generally larger than mitochondria and the internal membranes are less regularly arranged than the cristae of the mitochondria. The development of these bodies into mature plastids has been followed by a number of workers (e.g., 34) and will not be considered here.

(2) Mitochondria. Plant mitochondria have fine structure basically similar to that originally described in animal cells (38). They are usually spherical or spheroidal bodies bounded by two membranes, the inner of which shows numerous infoldings designated as cristae mitochondriales (Figs. 12–14). In general, mitochondria of meristematic plant cells show fewer cristae with less

regular arrangement than those of animal cells (Fig. 13). Between the cristae diffuse dense ground material is commonly seen (Fig. 12). Although usually regular in outline plant mitochondria may take on a variety of shapes (73). Mitochondria have been clearly demonstrated to be the centers of oxidative metabolism in cells (22), an extensive subject which cannot be discussed here.

The origin of mitochondria is unclear. Membrane-bounded bodies lacking internal organization have been reported by several workers (31, 54, 73) but it has not been convincingly demonstrated that these represent either young proplastids or promitochondria. The variety of irregular shapes which mature mitochondria often assume suggest division but again this remains unproved (73). Whaley *et al.* (73) review a number of papers which propose that mitochondria arise from either cytoplasmic bodies, Golgi-apparatus, nucleus, or plasma membrane, demonstrating the general uncertainty about the subject.

(3) Dictyosomes. Before the application of the electron microscope to cell structure the existence of dictyosomes (Golgi-apparati) in plant cells was controversial (35). This controversy has now been settled since a number of electron microscope studies have conclusively shown that meristematic plant cells commonly possess structures similar to the dictyosomes of animals (e.g., 43, 72). Essentially the individual dictyosome consists of a regular group of parallel, flattened, membranous sacs or cisternae with a number of small vesicles at their edges. The number of sacs varies but is usually fewer than 10. The membranes are similar to those of mitochondria and endoplasmic reticulum (below), but unlike the latter they appear never to have ribosomes associated with them, i.e., they are smooth-surfaced.

Both the origin and function of dictyosomes in plant cells are unknown. The associated vesicles are probably important in these respects but it is impossible to decide whether they represent bodies moving into or out of the cisternae. In the first case they might represent precursor material of the cisternae (25) while in the latter they could be "packages" of metabolic materials being released to the cytoplasm (72) after synthesis or accumulation in the cisternae.

(4) Endoplasmic reticulum. Unlike the above cytoplasmic structures which, to more or less extent, were known in the light microscope, the endoplasmic reticulum (ER) was essentially a new discovery (44) from electron microscopy. The ER is basically a system of membrane-bounded vesicles, tubules, and cisternae which permeates the cytoplasm, the individual membranes being similar in width and density to other membranes of the cytoplasm and usually occurring as double profiles enclosing a space about 150 Å wide (45). As stated in connection with nuclear structure the double nuclear membrane

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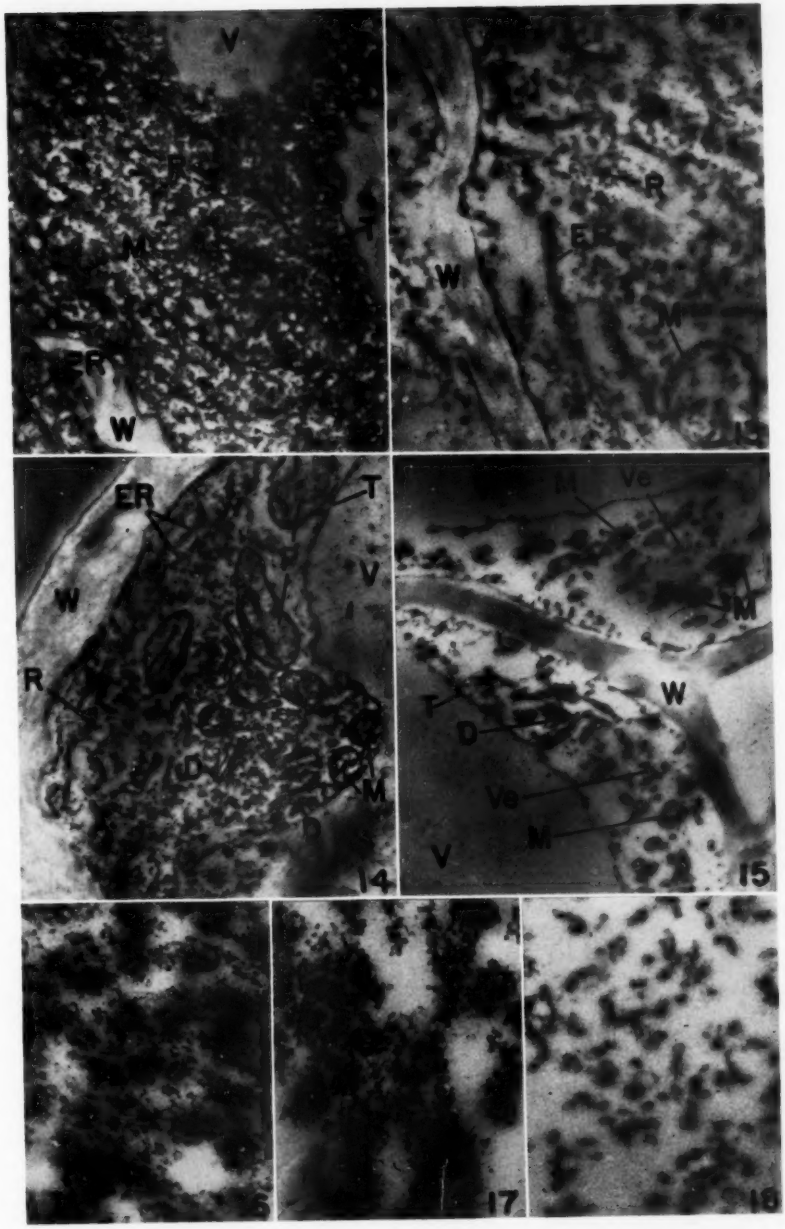
FIG. 12. Cytoplasm in cell from an oat coleoptile about 2 mm long. × 17,000
FIG. 13. As in Fig. 12, from a coleoptile 5 mm long. × 17,000
FIG. 14. As in Fig. 12, from a coleoptile 10–12 mm long. × 11,200
FIG. 15. As in Fig. 12, from a coleoptile about 35 mm long. × 10,000
FIG. 16. Portion of microsomal fraction from oat coleoptiles about 17 mm long. × 40,000
FIG. 17. As in Fig. 16, from coleoptiles about 34 mm long. × 40,000
FIG. 18. As in Fig. 16, from coleoptiles about 54 mm long. × 26,500



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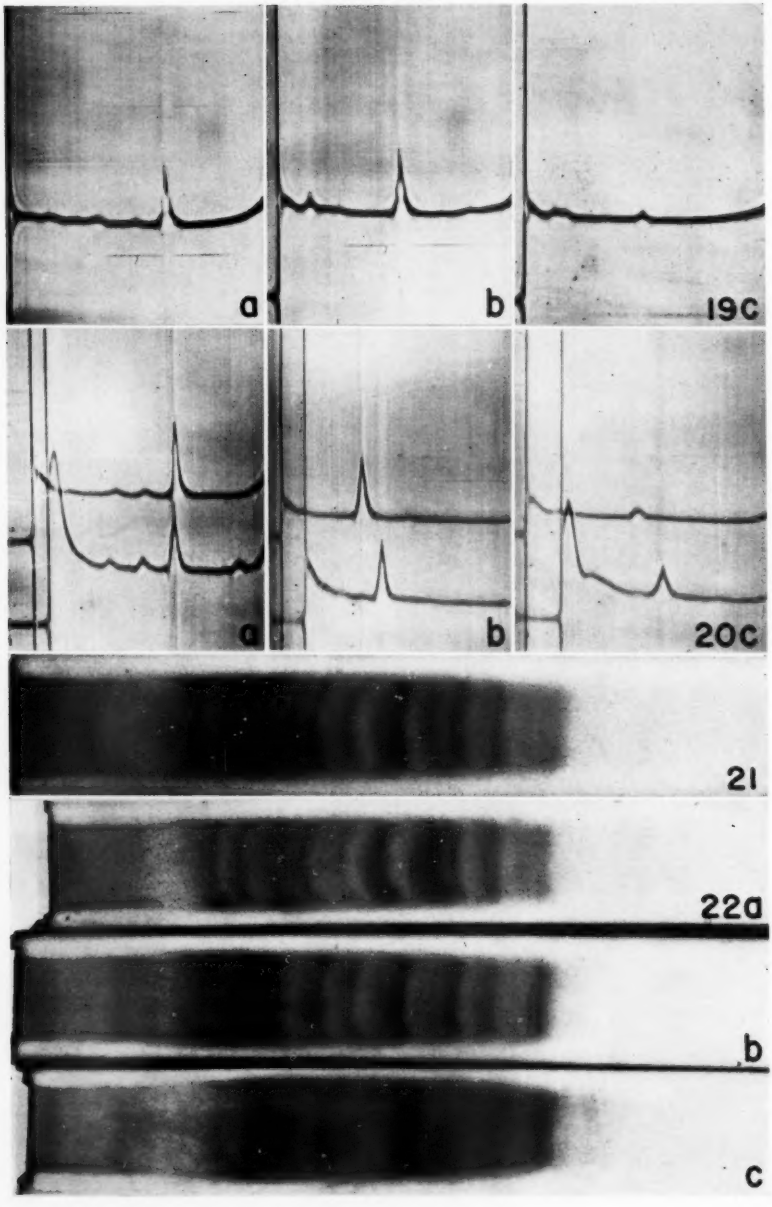

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may be regarded as a specialization of the ER (45, 71) and Whaley and co-workers (72, 74) have shown that in young cells of corn root there is a common association between elements of the ER and plasmodesmata. Thus in these young plant cells there appear to be regular channels from nucleus to nucleus via the ER. Such connections are probably of great significance in meristem development but their actual function is at present unknown.

Ribosomes (discussed below) often occur in close association with the ER, producing what are commonly known as rough-surfaced membranes. Since the ribosomes appear to be intimately involved in protein synthesis the ER has also been implicated in this process. Palade (39), for example, presented direct electron microscope evidence that, in pancreas, material similar to protein is formed within the cisternae of the ER. During cell fractionation the ER usually breaks up to form vesicles which sediment primarily in the microsomal fraction (40, 73), the fraction commonly found to be active in in vitro incorporation of amino acids into proteins (10).

(5) Ribosomes. The last generally important cytoplasmic organelles to be considered here are the ribosomes (microsomal particles, ribonucleoprotein particles). Unlike the previously described elements of the cytoplasm, which are primarily membranous, the ribosomes are small dense particles about 150 Å in diameter which may occur free in the cytoplasm or bound to membranes of the ER. The unbound particles are particularly abundant in young plant cells (31, 54) and are readily demonstrated, as shown by Watson (70), through "staining" with lead hydroxide (Fig. 8). Ribosomes of the same general size and composition have been revealed in all classes of organisms (10) and appear to be one of the few universal cytological structures.

Purified preparations of ribosomes are readily obtained by cell fractionation procedures and those from pea seedlings have been extensively analyzed by Ts'o and co-workers (63, 65). Briefly, they have shown these ribosomes to be particles of molecular weight 4 to 4.5 $\times$ 10^6 with a protein/RNA ratio of about 3/2. In the analytical ultracentrifuge they appear quite homogeneous (Fig. 19), with a sedimentation coefficient of about 80 *s*. Removal of magnesium from the particles causes them to dissociate into specific subunits with sedimentation coefficients 60 *s*, 40 *s*, and 25 *s*, but organization within the subunits is still largely unknown. A number of biochemical investigations (e.g., 46) which cannot be mentioned in detail here have indicated that ribosomes are active agents in the final stages of protein synthesis and they are thus bodies of great interest in considering growth and development.

FIG. 19. Schlieren patterns from ultracentrifugation of microsomal fractions prepared from samples of 250 oat coleoptiles. The main peak is the 76–80 *s* peak typical of ribosomes. Average lengths of coleoptiles were (*a*) 10 mm, (*b*) 30 mm, and (*c*) 54 mm (fully elongated).

FIG. 20. As in Fig. 19 except that a crude microsomal fraction was used (see text). The fractions giving the lower patterns were identical with those giving the upper patterns except that they were suspended in 0.3% desoxycholate instead of water. Average lengths of coleoptiles were (*a*) 15 mm, (*b*) 35 mm, and (*c*) 50 mm (fully elongated).

FIG. 21. Pattern from starch-gel electrophoresis of acid-soluble proteins from ribosomes of pea bud. Migration from left to right; cathode at right; pH 4.2, 0.02 *M* acetate buffer; amido black stain.

FIG. 22. As in Fig. 21, comparing proteins from ribosomes of various organs of pea seedlings: (*a*) bud, (*b*) etiolated stem, (*c*) root.

Cytoplasmic Structure in Elongating Cells

The above brief summary of cytoplasmic structure within plant cells refers, as noted, primarily to meristematic cells. It is obviously of considerable interest to learn of the behavior of the various elements during growth and Dr. S. T. Bayley and I have undertaken a study of cytoplasmic structure within elongating oat coleoptile cells. The investigation was carried out on tissue fixed in osmic acid, which has the disadvantage that in plant cells it only gives moderately good preservation of membranous elements (73) but is of value in that ribosomes are retained (45).

Figure 12 shows a portion of a cell from a coleoptile about 2 mm long. The appearance is typical of a young plant cell (31, 54), relatively little endoplasmic reticulum and large numbers of unbound ribosomes throughout the main cytoplasmic area giving a very dense background. The general changes which occur in this structure during elongation are indicated in Figs. 13–15, which show examples of cytoplasmic structure in coleoptile cells of increasing length to maturity. These changes may be briefly summarized as follows.

(1) Concerning mitochondria, there appeared to be a moderate tendency for the amount of internal material, both cristae and amorphous electron dense substance, to increase as growth proceeded (Fig. 15). The total number of mitochondria probably also increased since the number in any area remained about the same in all cells even though cell volume was greatly increased in the older coleoptiles (Figs. 14 and 15).

(2) Numerous prominent dictyosomes were evident throughout growth and the morphology remained essentially unchanged from that in young cells (Fig. 14). As with mitochondria, dictyosomes were readily visible in older cells indicating that the number must have increased as growth proceeded.

(3) Large amounts of endoplasmic reticulum were not common in these cells, even in the older ones, although isolated elements were always present. However, as growth proceeded there was clearly an increase in the amount of membranous material present in the cytoplasm. This material was usually irregularly vesicular and its significance is not clear. It may represent endoplasmic reticulum disrupted during preparation since vacuolated plant cells are particularly difficult to fix, or it may be a real component of the cells produced perhaps by the dictyosomes. Further work to clarify the nature of this membranous component of older cells is obviously essential, but these results clearly indicate that the amount of intracytoplasmic membrane substance increases markedly during cell expansion.

(4) The most striking structural change in the cytoplasm during cell growth concerned the ribosomes. The densely packed ribosomes of the young meristematic cells disappeared as elongation proceeded and in older cells free ribosomes were rarely visible. The result of this change was to give an apparent "empty" background in the electron micrographs of cytoplasm in older cells (Fig. 15).

From these electron microscope observations it was clear that the main change in the cytoplasmic organelles concerned structures which would enter the microsomal fraction obtained through cell fractionation, viz., the endoplasmic reticulum, small vesicular membranous elements, and ribosomes. To follow these changes further we prepared the microsomal fraction (65) from

coleoptiles of different ages, fixed the centrifugal pellets in osmic acid, and sectioned them for the electron microscope. Figures 16–18 show typical electron micrographs of areas within pellets from coleoptiles respectively 17, 37, and 54 mm long. As expected, the fraction from relatively young cells (Fig. 16) consisted almost entirely of ribosomes but somewhat surprisingly a similar situation was found in the fraction from the 37-mm coleoptiles (Fig. 17). Only the fraction from older cells (Fig. 18) showed a preponderance of membranous material. From these results it was clear that although the observations on sections of cells appeared to show a decrease in ribosomes with growth, this was probably a result of dilution due to cell expansion rather than actual loss of particles.

As a test of this dilution idea we prepared the microsomal fraction from individual samples of 250 coleoptiles of different lengths, made each preparation up to the same volume, and analyzed the preparations in the analytical ultracentrifuge. Some typical results are presented in Fig. 19, which shows that the size of the specific 80 *s* peak due to the ribosomes remained roughly constant during growth and only diminished as growth ceased. This result confirms that the number of ribosomes per coleoptile cell tended to remain constant throughout the period of elongation and the apparent loss of ribosomes in the in situ studies was only a dilution effect. The real loss of ribosomes at the cessation of elongation, as indicated by the ultracentrifuge analysis, may be a general phenomenon but it may also be a special effect in this tissue, which, not being part of the main plant axis, probably senesces early.

The above results refer only to ribosomes which are apparently not bound to membranes of ER in the cell and it is obviously of interest to obtain similar information on ribosomes which may be associated with the ER. Palade and Siekevitz (40) showed that in animal cells, ribosomes which are bound to membranes of the ER may be readily freed through treatment with desoxycholate (DOC). We therefore applied ultracentrifuge analyses to equal-volume crude "microsomal" fractions prepared with and without DOC treatment, on the expectation that any increase in the size of the 80 *s* peak due to DOC treatment would represent ribosomes freed from ER membranes. In these studies the so-called mitochondrial fraction (66) was included with the microsomal fraction so that no larger membranous elements with attached ribosomes would be lost in fractionation. Figure 20 shows some typical ultracentrifuge results from this type of study. DOC treatment of the fraction from actively elongating coleoptiles made little difference to the 80 *s* peak indicating the presence of few bound ribosomes in these preparations. This result agrees with the electron microscope observations on sections of microsomal fractions reported above. On the other hand, DOC treatment of microsomes from older coleoptiles, where the number of free ribosomes was greatly reduced, caused a significant increase in the size of the 80 *s* peak. Again this agreed with results from sections which revealed primarily vesicular material in microsomal preparations from older coleoptiles.

In summary, these studies on ribosomes in developing coleoptile cells indicate that the number of unbound ribosomes per cell remains roughly constant throughout the elongation period and then declines sharply at the cessation of growth. On the other hand, there are relatively few ribosomes

attached to the ER in growing cells and only in older cells could a significant number be detected. There is thus a noticeable change in the ribosomal composition of these plant cells associated with growth, which undoubtedly reflects a change in the function of these components. A rather similar picture concerning the ribosomes and ER has been noted by Lund *et al.* (31) in the development of corn roots, suggesting that the transformations observed here are of general significance in plant cell development. A tempting hypothesis might be that the free ribosomes, preponderant in growing cells, are primarily concerned with synthesis of structural protein of the cytoplasm while the ribosomes bound to membranes are basically concerned with production of metabolic proteins utilized in the function of the mature cell. A more thorough presentation and discussion of these results is intended in the near future (3).

Ribosome Composition

Apart from the primarily quantitative data on ribosome content presented above, the qualitative nature of ribosomes is of considerable interest in relation to developmental physiology. If the particles are centrally involved in protein synthesis (10) it is possible that they will show changes in composition which would reflect the changes in metabolism associated with cell differentiation. Surprisingly, however, by most techniques ribosomes show relatively little heterogeneity. For example, particles from different species (64) and different organs of the same species (65) show similar ultracentrifuge behavior. The RNA to protein ratios are also generally consistent and even the amino acid composition of ribosomes from animal and plant sources is remarkably similar (64). Wright (76) has noted some antigenic differences in oat microsomes which appear to be associated with differentiation but the basis of these differences is unclear.

Recently, we have shown that the ribosomes of apical buds of pea seedling contain a complex group of weakly basic proteins which are soluble in acid (53). Figure 21 shows a typical pattern obtained from electrophoresis of these proteins in starch-gel. In the hope that this complex group of proteins would reflect changes in the ribosomes associated with different tissues we have prepared a similar protein fraction from ribosomes of several organs of the pea and subjected them to starch-gel electrophoresis. Figure 22 shows electrophoretic patterns comparing proteins derived from ribosomes of pea bud, stem, and root and it can be seen that in the main the patterns are similar. These results are quite preliminary and need extension but at the moment they suggest that the basic protein complex is a consistent feature of the ribosomes throughout the plant, rather than a differentiating element. The significance of such a result is open to many interpretations and must be regarded with hesitancy. For example, these proteins may only represent part of a structural shell of the particles which is not directly involved in their specific functions. On the other hand, the similarity of the proteins may reflect the fact that the ribosomes, although from different tissues, were all primarily derived from parenchyma cells and for this reason were basically similar in composition and function. Clearly further work on the qualitative nature of ribosomes is essential in interpreting developmental processes.

The Cell Wall

Any discussion of structure of plant cells in relation to developmental physiology must take cell walls into consideration These prominent structures determine the gross form of most plant cells and are therefore basically responsible for the morphological characteristics of plant tissues and organs. The problem of growth and morphological differentiation of plants is therefore in large measure a problem in understanding growth and development of cell walls.

The most important consideration in our present views of the behavior of cell walls comes from the recognition that walls, although appearing essentially homogeneous in the bright field microscope, contain an organized substructure. Thus, for example, it was recognized many years ago from studies with polarized light that walls contain a crystalline component of varying organization which persists after extensive extraction of wall material. In recent years this structural situation has been clarified through chemical and X-ray diffraction analyses and electron microscope observations, and plant cell walls have been shown to have a universally similar organization, viz., a continuous amorphous matrix composed primarily of polysaccharides, in which long crystalline microfibrils of cellulose, or chitin in some lower forms, are embedded (29). The relation of these two structural phases of the wall to growth and development will be briefly discussed below.

The Wall Matrix

No simple organized structure has been demonstrated in the polysaccharide matrix of walls but certainly the molecules must interact to produce a three-dimensional network which forms the continuous phase of the wall. As the continuous phase the matrix then takes on significance in determining rigidity of the wall, thereby possibly actively influencing processes of cell expansion.

In the 1930's Heyn (24) clearly demonstrated that auxin could produce changes in plasticity of primary walls and suggested that this controlled plasticization could form the basis of a growth-regulating mechanism. In recent years Heyn's ideas have gained popularity, and attention has been turned to the nature of the changes within the matrix which, through auxin mediation, could regulate plasticity of walls. The most popular and attractive theory, suggested by Bennet-Clark (5), involves controlled changes in the amount of cross linking between carboxyl groups of pectic acid, primarily through the medium of calcium bridges. Some of the evidence in favor of this hypothesis is that added calcium is a strong inhibitor of cell expansion (5) and reduces wall plasticity (61), chelating agents often stimulate cell expansion (5), pectin methylesterase enzyme shows responses to auxin (12, 20), and some effects of auxin on incorporation of isotope into the methyl esters of pectic substance have been demonstrated (37). Unfortunately, a review of recent studies (52) shows that in spite of this indirect evidence the pectin-bridge hypothesis remains unsubstantiated. For example, auxin-stimulated pectin methylesterase responses (26) and isotope incorporation into pectin methyl-ester from methionine (15) do not appear to be general reactions, and calcium levels in walls are unaffected by either auxin (16) or chelating agents (13).

Recent chemical analyses indicate that the main bulk of the matrix in oat coleoptiles consists of hemicelluloses and it has been suggested that these compounds may control wall rigidity (7). Unfortunately the hemicelluloses do not possess an obvious capacity for reaction as do the pectic substances and isotope incorporation studies have, to date, not revealed an obvious response of hemicelluloses to growth substance. The matrix of the wall therefore remains a possible important center of growth control but the biochemical nature of such reaction is unknown.

Microfibrillar Arrangement

Apart from a possible role in controlling cell expansion, as noted above, walls are of basic importance in determining the form and structural characteristics of cells. This aspect of walls is in turn largely dependent on the semirigid microfibrillar component which functions somewhat as the steel network within reinforced concrete. Obviously the factors which control number, orientation, and arrangement of microfibrils within the wall are of considerable significance in cell development and differentiation.

Early in the study of organization within expanding walls one common structural plan became evident; in thin-walled parenchyma cells the majority of microfibrils were shown to be oriented more or less transversely to the cell axis producing what Frey-Wyssling (19) termed "tubular texture". In the last few years several studies have led to a partial explanation of how such a structure is developed and maintained. First, marker experiments (14) and studies on pit-field distribution (67) showed that walls in typical primary tissue grow by expansion over the entire surface rather than by localized expansion at cell tips. As a corollary, autoradiographic studies (9, 49, 51, 68) indicated that in such cells deposition of microfibrils also occurs evenly over the entire wall. Second, it was recognized by Roelofsen and Houwink (47) that in cells with tubular texture the microfibrils on the inner surface were closely oriented in the transverse direction while those on the outer surface showed considerable disorientation, with numbers approaching longitudinal. From this observation these authors proposed the multinet theory of wall growth, which assumes that microfibrils are deposited at the protoplasmic surface in the transverse direction and through cell elongation are gradually pulled into an approximately longitudinal orientation while at the same time being thinned out. As growth proceeds, new transverse microfibrils are con-

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FIGS. 23–27. Transverse sections of walls of cortical cells in elongation zone of bean stems. Walls extracted with pectinase to reveal cellulose microfibrils. Embedding medium removed, sections shadowed with palladium–gold. Arrows point toward centers of cell lumens.

FIG. 23. Junction of three cells near outside of cortex. Note the multilayers of longitudinal microfibrils. × 8000

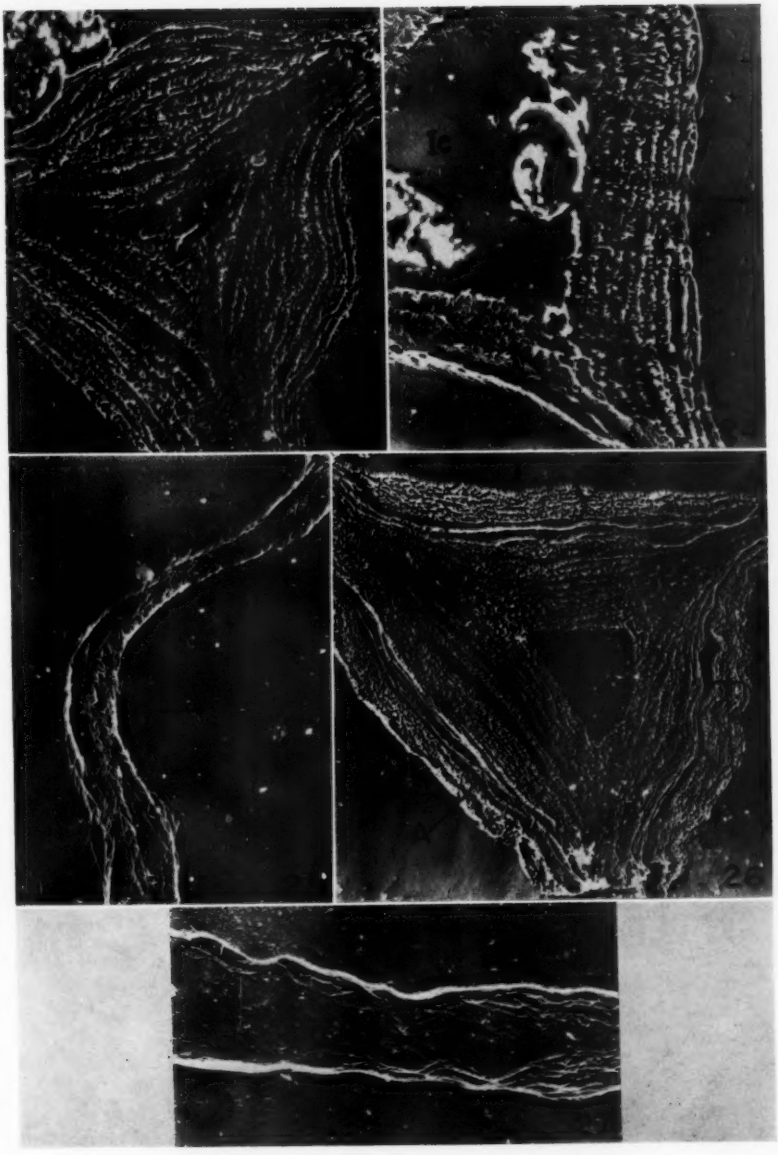
FIG. 24. As in Fig. 23, deeper in cortex. Fewer layers of longitudinal microfibrils present. × 10,500

FIG. 25. Walls of two cells deep in the cortex. These are typical parenchyma walls. × 10,500

FIG. 26. Junction of three cells on the upper side of a stem under horizontal stress. Apart from the normal layers of longitudinal microfibrils, as in Fig. 1, numerous extra layers have been added on the inside (A). × 5000

FIG. 27. Walls from lower side of a stem under horizontal stress. × 7500

PLATE V



Setterfield—Can. J. Botany

tinually deposited on the inside to compensate for the thinning, thereby giving a steady state situation in which transverse microfibrils predominate and the wall continually shows negative birefringence. Multinet structure has been shown to be present in several types of hair cells (47) and parenchyma cells (49, 50, 68, 69) and has been elegantly demonstrated by Green in the thickened walls of *Nitella* (23), which appear to be an extreme case. Thus the multinet mechanism seems of general applicability to regions of growing walls which show negative birefringence and tubular texture.

Not all regions of growing walls are of this type, however. Majumdar and Preston (32) showed years ago with the polarizing microscope that the thickened primary walls of collenchyma cells have longitudinally oriented cellulose predominating. We have performed electron microscope analyses on both collenchyma (4) and epidermal walls (2) and have shown that a common feature of thickened primary walls is the presence of multilayers of longitudinal microfibrils. In addition, several workers have recently shown that longitudinal ribs of microfibrils are present on the outer surface of parenchyma cells and are part of the primary wall (9, 50, 69). There is thus a range of primary wall types from those showing almost pure multinet structure to those showing predominately organized layers of longitudinal microfibrils. This range of primary wall structure is well illustrated by the cortical cells in young bean stems where, as shown in Figs. 23–25, the outer cells show considerable numbers of layers of longitudinal microfibrils while the cells deeper in the cortex show progressively fewer longitudinal microfibrils and approach the structure of simple parenchyma with predominant multinet structure and a few single-layered ribs of longitudinal microfibrils. These results indicate that the form of primary walls is in large measure dependent on the relative balance between the transverse and longitudinal microfibrils present.

This consideration focusses attention on the central outstanding problem of wall structure and development, the nature of the mechanisms controlling microfibril deposition. At present there is essentially no direct knowledge of either the biochemical processes responsible for microfibril formation or the mechanisms which control the orientation of microfibrils as they are deposited. The latter process undoubtedly involves, at least in part, organization within the cytoplasm but apparently this organization must be at a level not revealed by present electron microscope techniques since no obvious relation between cytoplasmic organelles and wall structure has yet been demonstrated (51).

One obvious approach to study of processes controlling microfibril deposition is through experimental modification of the normal system. Unfortunately, so far this approach has proved unexpectedly difficult since deposition and elongation seem closely coupled and interference with one process generally upsets the other (52). We have, however, examined one system in which some modification of wall organization is possible. As described by Boysen-Jensen (11), if a young bean stem is grown horizontally in a glass tube which prevents upward bending, the cortical cells on the lower side expand radially and retain their wall thickness while those on the upper side remain about the same diameter but develop thicker walls. An electron microscope analysis of the microfibrillar arrangement in these walls shows that in the cells on the lower side of the horizontal stem microfibrils more or less transverse to the

cell axis are being deposited (Fig. 27) while on the upper side numerous layers of longitudinal microfibrils appear (Fig. 26). The direction of deposition in both areas may be reversed by rotating the confined stem through 180°. Here then, direction of microfibril deposition is directly influenced by an experimental treatment but unfortunately interpretation is difficult. Probably the differential deposition of microfibrils represents an indirect response of the cells to the complex situation in the confined horizontal stem. In response to auxin-stimulated expansion on the lower side transverse microfibrils are laid down while on the upper side, which is subjected to extreme bending stresses, the cells respond by adding stiffening layers of longitudinal microfibrils. The key question remains, however, what is the means of such responses? We have attempted to detect differences in cytoplasmic organization on the two sides of the stem but, owing to generally poor fixation of these vacuolate cells, we have been unsuccessful. Thus the central problem of microfibril deposition remains unanswered and will probably require new approaches to cytoplasm–wall interaction for elucidation.

Conclusion

As stated in the Introduction the purpose of this paper was to present some evidence which bears on the relationship between structure in plant cells and physiological processes involved in growth and development. It becomes obvious, however, that although advances have been made in recent years we are still far from any general understanding of the integrated function of cell organelles in developmental processes, and probably the main value of this discussion has been to point up a few of the problems in plant-cell physiology relating to growth. The specific problems raised may be briefly summarized as follows:

(1) Unlike the situation with other cell organelles, of which the fine structure is reasonably well known, the organization within the nucleus remains essentially undescribed. It is thus impossible at present to give very meaningful structural interpretation to the various metabolic functions proposed for the nucleus and nucleolus. Possibly new methods of fixation and electron staining will help clarify this situation.

(2) Apart from the structural situation within the nucleus the physiological processes which regulate cell division are also of direct importance in cell and tissue development. The results presented here suggest that several different mechanisms of control may exist, but at present the actual biochemical reactions involved are unknown.

(3) Concerning the cytoplasm, although the structure and function of the major organelles, the plastids and mitochondria, are reasonably well known, the physiological role of the remaining prominent structures, dictyosomes, endoplasmic reticulum, vesicular membranes, and ribosomes, is still problematic. The results presented indicate that these latter elements undergo considerable change as cell enlargement proceeds and the physiological consequences of these changes are probably of significance in developmental processes.

(4) The polysaccharide matrix of primary cell walls appears as a possible site

of growth regulation through control of wall plasticity. However, the chemical basis of such regulation remains unknown and more rigorous chemical study than that undertaken to date will probably be required before this problem yields.

(5) Arrangement of microfibrils within walls is now fairly well understood but the processes responsible for this arrangement are obscure. This problem involves both the biochemical reactions involved in microfibril production and a type of cytoplasmic organization involved in microfibril orientation which has not yet been appreciated.

References

1. AMANO, M. and LEBLOND, C. P. 1960. Comparison of the specific activity time curves of ribonucleic acid in chromatin, nucleolus and cytoplasm. Exptl. Cell Research, **20**, 250–253.
2. BAYLEY, S. T., COLVIN, J. R., COOPER, F. P., and MARTIN-SMITH, C. A. 1957. The structure of the primary epidermal cell wall of Avena coleoptiles. J. Biophys. Biochem. Cytol. **3**, 171–182.
3. BAYLEY, S. T. and SETTERFIELD, G. In preparation.
4. BEER, M. and SETTERFIELD, G. 1958. Fine structure in thickened primary walls of collenchyma cells of celery petioles. Am. J. Botany, **45**, 571–580.
5. BENNET-CLARK, T. A. Salt accumulation and mode of action of auxin. A preliminary hypothesis. *In* The chemistry and mode of action of plant growth substances. *Edited by* R. L. Wain and F. Wightman, Butterworth Scientific Publications, London. pp. 284–291.
6. BENNETT, H. S. 1959. Fine structure of cell nucleus, chromosomes, nucleoli and membrane. *In* Biophysical science – A study program. *Edited by* J. L. Oncley. John Wiley & Sons, Inc., New York. pp. 297–300.
7. BISHOP, C. T., BAYLEY, S. T., and SETTERFIELD, G. 1958. Chemical constitution of the primary cell walls of Avena coleoptiles. Plant Physiol. **33**, 283–289.
8. BISWAS, B. B. and SEN, S. P. 1959. Relationship between auxins and nucleic acid synthesis in coleoptile tissues. Nature, **183**, 1824–1825.
9. BÖHMER, H. 1958. Untersuchungen über das Wachstum und den Feinbau der Zellwände in der Avena-Koleoptile. Planta, **50**, 461–497.
10. BONNER, J. 1959. Protein synthesis and the control of plant processes. Am. J. Botany, **46**, 58–62.
11. BOYSEN-JENSEN, P. 1958. Untersuchungen über Determination und Differenzierung. 5. Über die Wirkungsweise des Wuchstoffes in dem Epikotyl von *Phaseolus*. Kgl. Danske Videnskab. Selskab, Biol. Medd. **23**,(8), 1–51.
12. BRYAN, W. H. and NEWCOMB, E. H. 1954. Stimulation of pectin methylesterase activity of cultured tobacco pith by indoleacetic acid. Physiol. Plantarum, **7**, 290–297.
13. CARR, D. J. and NG, E. K. 1959. The sequestration of calcium from preparations of wheat coleoptile cell walls. Physiol. Plantarum, **12**, 264–274.
14. CASTLE, E. S. 1955. The mode of growth of epidermal cells of the Avena coleoptile. Proc. Natl. Acad. Sci. U.S. **41**, 197–199.
15. CLELAND, R. 1960. Auxin-induced methylation in maize. Nature, **185**, 44.
16. CLELAND, R. 1960. Effect of auxin upon loss of calcium from cell walls. Plant Physiol. **35**, 581–584.
17. ESAU, K. 1953. Plant anatomy. John Wiley & Sons, Inc., New York.
18. FRENSTER, J. H., ALLFREY, V. G., and MIRSKY, A. E. 1960. Metabolism and morphology of ribonucleoprotein particles from the cell nucleus of lymphocytes. Proc. Natl. Acad. Sci. U.S. **46**, 432–444.
19. FREY-WYSSLING, A. 1953. Submicroscopic morphology of protoplasm. Elsevier Publishing Co., Amsterdam.
20. GLASZIOU, K. T. and INGLIS, S. D. 1958. The effect of auxins on the binding of pectin methylesterase to cell walls. Australian J. Biol. Sci. **11**, 127–141.
21. GOLDSTEIN, L. and MICOU, J. 1959. On the primary site of nuclear RNA synthesis. J. Biophys. Biochem. Cytol. **6**, 301–303.
22. GREEN, D. E. 1958. Mitochondrial structure and function. *In* Subcellular particles. *Edited by* T. Hayashi. The Ronald Press Co., New York. pp. 84–103.
23. GREEN, P. B. 1960. Multinet growth in the cell wall of *Nitella*. J. Biophys. Biochem. Cytol. **7**, 289–296.

24. HEYN, A. N. J. 1940. The physiology of cell elongation. Botan. Rev. **6**, 515–574.
25. HODGE, A. J., MCLEAN, J. D., and MERCER, F. V. 1956. A possible mechanism for the morphogenesis of lamellar systems in plant cells. J. Biophys. Biochem. Cytol. **2**, 597–608.
26. JANSEN, E. F., JANG, R., and BONNER, J. 1960. Binding of enzymes to Avena coleoptile cell walls. Plant Physiol. **35**, 567–574.
27. JENSEN, W. A., KAVALJIAN, L. G., and MARTINOT, S. 1960. The incorporation of ^{3}H-thymidine by developing root tip cells. Exptl. Cell Research, **20**, 361–367.
28. JOHNSTON, F. B., SETTERFIELD, G., and STERN, H. 1959. The isolation of nucleoli from ungerminated pea embryos. J. Biophys. Biochem. Cytol. **6**, 53–66.
29. KERR, T. 1951. Growth and structure of the primary wall. *In* Plant growth substances. *Edited by* F. Skoog. University of Wisconsin Press, Madison, Wis. pp. 37–42.
30. LAFONTAINE, J. G. 1958. Structure and mode of formation of the nucleolus in meristematic cells of *Vicia faba* and *Allium cepa*. J. Biophys. Biochem. Cytol. **4**, 777–784.
31. LUND, H. A., VATTER, A. E., and HANSON, J. B. 1958. Biochemical and cytological changes accompanying growth and differentiation in the roots of *Zea mays*. J. Biophys. Biochem. Cytol. **4**, 87–98.
32. MAJUMDAR, G. P. and PRESTON, R. D. 1941. The fine structure of collenchyma cells in *Heracleum Sphondylium L.* Proc. Roy. Soc. B, **130**, 201–217.
33. MOSES, M. J. and LAFONTAINE, J. G. 1959. Components of the nucleus at interphase and during division. Proc. Intern. Botan. Congr. 9th Congr. Montreal. (Abstr.)
34. MÜHLETHALER, K. and FREY-WYSSLING, A. 1959. Entwicklung und struktur der proplastiden. J. Biophys. Biochem. Cytol. **6**, 507–512.
35. NAHM, L. J. 1940. The problem of golgi material in plant cells. Botan. Rev. **6**, 49–72.
36. NAYLOR, J. M. 1958. Control of nuclear processes by auxin in axillary buds of *Tradescantia paludosa*. Can. J. Botany, **36**, 221–232.
37. ORDIN, L., CLELAND, R., and BONNER, J. 1957. Methyl esterification of cell wall constituents under the influence of auxin. Plant Physiol. **32**, 207–212.
38. PALADE, G. E. 1952. The fine structure of mitochondria. Anat. Record, **114**, 427–444.
39. PALADE, G. E. 1956. Intracisternal granules in the exocrine cells of the pancreas. J. Biophys. Biochem. Cytol. **2**, 417–422.
40. PALADE, G. E. and SIEKEVITZ, P. 1956. Pancreatic microsomes. An integrated morphological and biochemical study. J. Biophys. Biochem. Cytol. **2**, 671–690.
41. PATAU, K., DAS, N. K., and SKOOG, F. 1957. Induction of DNA synthesis by kinetin and indoleacetic acid in excised tobacco pith tissue. Physiol. Plantarum, **10**, 949–966.
42. PATAU, K. and SWIFT, H. 1953. The DNA-content (Feulgen) of nuclei during mitosis in a root tip of onion. Chromosoma, **6**, 149–169.
43. PERNER, E. S. 1958. Elektronenmikroskopische Untersuchungen zur Cytomorphologie des sogenannten "Golgisystems" in Wurzelzellen verschiedener Angiospermen. Protoplasma, **49**, 407–446.
44. PORTER, K. R. 1953. Observations on a submicroscopic basophillic component of cytoplasm. J. Exptl. Med. **97**, 727–750.
45. PORTER, K. R. and MACHADO, R. D. 1960. Studies on the endoplasmic reticulum IV. Its form and distribution during mitosis in cells of onion root tip. J. Biophys. Biochem. Cytol. **7**, 167–180.
46. RAAKE, I. D. 1959. Studies on protein synthesis with ribonucleoprotein particles from pea seedlings. Biochim. et Biophys. Acta, **34**, 1–9.
47. ROELOFSEN, P. A. and HOUWINK, A. L. 1953. Architecture and growth of the primary cell wall in some plant hairs and in the *Phycomyces* sporangiophore. Acta Botan. Neerl. **2**, 218–225.
48. SEED, J. 1960. Inhibition of nucleic acid synthesis caused by X-irradiation of the nucleolus. Proc. Roy. Soc. B, **152**, 387–396.
49. SETTERFIELD, G. and BAYLEY, S. T. 1957. Studies on the mechanism of deposition and extension of primary cell walls. Can. J. Botany, **35**, 435–444.
50. SETTERFIELD, G. and BAYLEY, S. T. 1958. Arrangement of cellulose microfibrils in walls of elongating parenchyma cells. J. Biophys. Biochem. Cytol. **4**, 377–382.
51. SETTERFIELD, G. and BAYLEY, S. T. 1959. Deposition of cell walls in oat coleoptiles. Can. J. Botany, **37**, 861–870.
52. SETTERFIELD, G. and BAYLEY, S. T. 1961. Structure and physiology of cell walls. Ann. Rev. Plant Physiol. **12**, In press.
53. SETTERFIELD, G., NEELIN, J. M., NEELIN, E. M., and BAYLEY, S. T. 1960. Studies on basic proteins from ribosomes of buds of pea seedlings. J. Mol. Biol. **2**, In press.
54. SETTERFIELD, G., STERN, H., and JOHNSTON, F. B. 1959. Fine structure in cells of pea and wheat embryos. Can. J. Botany, **37**, 65–72.
55. SIRLIN, J. L. 1960. The nucleolus problem. Nature, **186**, 275–278.

56. STERN, H., JOHNSTON, F. B., and SETTERFIELD, G. 1959. Some chemical properties of isolated pea nucleoli. J. Biophys. Biochem. Cytol. **6**, 57–60.
57. STYLES, D. and SETTERFIELD, G. Unpublished data.
58. SWIFT, H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. Proc. Natl. Acad. Sci U.S. **36**, 643–654.
59. SWIFT, H. 1959. Studies on nuclear fine structure. Brookhaven Symposia in Biol. No. 12, 134–152.
60. SWIFT, H. 1959. Studies on nucleolar function. *In* A symposium on molecular biology. *Edited by* R. E. Zirkle. University of Chicago Press, Chicago. pp. 266–293.
61. TAGAWA, T. and BONNER, J. 1957. Mechanical properties of the Avena coleoptile as related to auxin and to ionic interactions. Plant Physiol. **32**, 207–212.
62. THIMANN, K. V. 1960. Plant growth. *In* Fundamental aspects of normal and malignant growth. *Edited by* W. W. Nowinski. Elsevier Publishing Co., Amsterdam. pp. 748–822.
63. TS'O, P. O. P. 1958. Structure of microsomal nucleoprotein particles from pea seedlings. *In* Microsomal particles and protein synthesis. *Edited by* R. B. Roberts. Pergamon Press, New York. pp. 156–168.
64. TS'O, P. O. P., BONNER, J., and DINTZIS, H. 1958. On the similarity of amino acid composition of microsomal nucleoprotein particles. Arch. Biochem. Biophys. **76**, 225–234.
65. TS'O, P. O. P., BONNER, J., and VINOGRAD, J. Microsomal nucleoprotein particles from pea seedlings. J. Biophys. Biochem. Cytol. **2**, 451–466.
66. TS'O, P. O. P. and SATO, C. S. 1959. Synthesis of ribonucleic acid in plants I. Distribution of ribonucleic acid and of protein among subcellular components of pea epicotyls. Exptl. Cell Research, **17**, 227–236.
67. WARDROP, A. B. 1955. The mechanism of surface growth in parenchyma of Avena coleoptiles. Australian J. Botany, **3**, 137–148.
68. WARDROP, A. B. 1956. The nature of surface growth in plant cells. Australian J. Botany, **4**, 193–199.
69. WARDROP, A. B. and CRONSHAW, J. 1958. Changes in cell wall organization resulting from surface growth in parenchyma of oat coleoptiles. Australian J. Botany, **6**, 89–95.
70. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals II. Application of solutions containing lead and barium. J. Biophys. Biochem. Cytol. **4**, 727–730.
71. WATSON, M. L. 1959. Further observations on the nuclear envelope of the animal cell. J. Biophys. Biochem. Cytol. **6**, 147–156.
72. WHALEY, W. G., MOLLENHAUER, H. H., and KEPHART, J. E. 1959. The endoplasmic reticulum and the golgi structures in maize root cells. J. Biophys. Biochem. Cytol. **5**, 501–506.
73. WHALEY, W. G., MOLLENHAUER, H. H., and LEECH, J. H. 1960. The ultrastructure of the meristematic cell. Am. J. Botany, **47**, 401–449.
74. WHALEY, W. G., MOLLENHAUER, H. H., and LEECH, J. H. 1960. Some observations on the nuclear envelope. J. Biophys. Biochem. Cytol. **8**, 233–245.
75. WOODS, P. S. and TAYLOR, J. H. 1959. Studies of ribonucleic acid metabolism with tritium-labeled cytidine. Lab. Invest. **8**, 309–318.
76. WRIGHT, S. T. C. 1960. Occurrence of an organ-specific antigen associated with the microsomal fraction of plant cells and its possible significance in the process of cellular differentiation. Nature, **185**, 82–85.

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Contents

Page

F. L. Raymond and J. Reid—Dieback of balsam fir in Ontario 233

Stewart A. Brown—Studies of lignin biosynthesis using isotopic carbon. IX. Taxonomic distribution of the ability to utilize tyrosine in lignification . 253

J. J. Miller and James Reid—Stimulation by light of sporulation in *Trichoderma lignorum* (Tode) Harz 259

R. Rohringer and R. Heitefuss—Incorporation of P^{32} into ribonucleic acid of rusted wheat leaves 263

Gerald A. Mulligan and Raymond J. Moore—Natural selection among hybrids between *Carduus acanthoides* and *C. nutans* in Ontario 269

J. M. Naylor and G. M. Simpson—Dormancy studies in seed of *Avena fatua*. 2. A gibberellin-sensitive inhibitory mechanism in the embryo 281

E. W. B. Ward, J. B. Lebeau, and M. W. Cormack—Grouping of isolates of a low-temperature basidiomycete on the basis of cultural behavior and pathogenicity 297

Margaret E. Barr—Northern pyrenomycetes. II. Gaspesian Park 307

A. Stanley Holt—Further evidence of the relation between 2-desvinyl-2-formyl-chlorophyll-*a* and chlorophyll-*d* 327

Eville Gorham—Chlorophyll derivatives, sulphur, and carbon in sediment cores from two English lakes 333

D. J. Wort and B. C. Loughman—The effect of 3-amino-1,2,4-triazole on the uptake, retention, distribution, and utilization of labelled phosphorus by young barley plants 339

Constance A. Bowerman—*Lycoperdon* in eastern Canada with special reference to the Ottawa district 353

Dorothy F. Forward and Norah J. Nolan—Growth and morphogenesis in the Canadian forest species. IV. Radial growth in branches and main axis of *Pinus resinosa* Ait. under conditions of open growth, suppression, and release 385

Dorothy F. Forward and Norah J. Nolan—Growth and morphogenesis in the Canadian forest species. V. Further studies of wood growth in branches and main axis of *Pinus resinosa* Ait. under conditions of open growth, suppression, and release 411

Notes:

C. D. Taper and W. Leach—Note on the effects of calcium concentration in culture solutions upon the leaf and whole plant absorption of iron and manganese by dwarf kidney bean 437

G. H. DUFF MEMORIAL SYMPOSIUM ON DEVELOPMENTAL PHYSIOLOGY

G. Krotkov—Introductory remarks by the Chairman 439

F. C. Steward—Vistas in plant physiology: Problems of organization, growth, and morphogenesis 441

E. Bünning—Endogenous rhythms and morphogenesis 461

George Setterfield—Structure and composition of plant-cell organelles in relation to growth and development 469